Mature transforming growth factor-β (TGF-β) is proteolytically derived from the C terminus of a precursor protein. Latency-associated protein (LAP), the N-terminal remnant of the TGF-β precursor, is able to bind and neutralize TGF-β. Mature TGF-β exerts its activity by binding and complexing members of two subfamilies of receptors, the type I and II receptors. In addition to these signaling receptors, TGF-β can also interact with an accessory receptor termed the type III receptor. Using a surface plasmon resonance-based biosensor (BIAcore), we determined the mechanisms of interaction of four binding proteins (LAP, the type II and III receptor ectodomains (EDs), and a type II receptor ED/Fc chimera) with three TGF-β isoforms, and we quantified their related kinetic parameters. Using global fitting based on a numerical integration data analysis method, we demonstrated that LAP and the type II receptor/Fc chimera interacted with the TGF-β isoforms with a 1:1 stoichiometry. In contrast, the type II ED interactions with TGF-β were best fit by a kinetic model assuming the presence of two independent binding sites on the ligand molecule. We also showed that the type III ED bound two TGF-β molecules. Further experiments revealed that LAP was able to block the interactions of TGF-β with the two EDs, but that the two EDs did not compete or cooperate with each other. Together, these results strongly support the existence of a cell-surface complex consisting of one type III receptor, two TGF-β molecules, and four type II receptors, prior to the recruitment of the type I receptor for signal transduction. Additionally, our results indicate that the apparent dissociation rate constants are more predictive of the neutralizing potency of these TGF-β-binding proteins (LAP, the type II and III receptor EDs, and the type II receptor/Fc chimera) than the apparent equilibrium constants.

Transfoming growth factor-β (TGF-β)1 belongs to a family of peptides involved in the regulation of growth, development, tissue repair, tumorigenesis, inflammation, and host defense (1–4). Three isoforms (termed TGF-β1, TGF-β2, and TGF-β3) are present in mammalian cells. Mature TGF-β isoforms correspond to the carboxyl-terminal domain of a precursor protein termed prepro-TGF-β. Cleavage by a proconvertase yields both mature TGF-β and a prodomain called the latency-associated protein (LAP). Both TGF-β and LAP are disulfide-bonded homodimers and have been shown to remain noncovalently associated with each other after cleavage (5). This complex is inactive (latent), as it is not recognized by the TGF-β signaling receptors.

Mature TGF-β signals by binding and complexing two receptors, the type I and II receptors (6). Once this heteromeric receptor-ligand complex is formed, the type II receptor phosphorylates the type I receptor, and the signal is then translocated to the nucleus by members of the SMAD family (7, 8). In addition to the signaling receptors, TGF-β can also interact with a receptor known as the type III receptor that apparently does not signal. This receptor is thought to act as an enhancer of TGF-β activity by promoting its access to the signaling receptors (especially for the TGF-β2 isoform, which has a low affinity for the type II receptor) (9, 10). The type III receptor possesses two ligand-binding domains, one located in the N-terminal region and one in the C-terminal region of its ectodomain (11–14). However, it remains unclear if one or two TGF-β molecules bind to the type III receptor. The isolated ectodomains (EDs) of the type II and III receptors are soluble and retain their ability to bind to TGF-β isoforms (15–17). They exert, similarly to LAP, an antagonistic activity by binding to TGF-β and sequestering it away from cell-surface receptors (15, 18).

The purpose of this study was to determine the binding mechanisms of these three proteins, LAP and the type II and III EDs, in addition to a type II receptor ectodomain/Fc chimera with the three mammalian TGF-β isoforms. To quantify the kinetic parameters of these interactions, we used the BIAcore, a surface plasmon resonance-based biosensor that allows real-time measurement of macromolecular interactions. We demonstrated the following. 1) LAP interacts with all the TGF-β isoforms according to a conformational change model and a 1:1 stoichiometry. 2) TGF-β1 and TGF-β3 interactions with the type II ED are best fit by a kinetic model assuming the presence of two independent binding sites on the ligand molecule for the type II ED. In contrast, the interactions of these TGF-β isoforms with a type II ED/Fc chimera are best depicted by a rearrangement model and a 1:1 stoichiometry. 3) The type III isoforms according to a conformational change model and a 1:1 stoichiometry.
ED binds two TGF-β molecules. 4) LAP is able to block the interactions of TGF-β with the two ectodomains. 5) Both ectodomains do not compete or cooperate with each other when binding to TGF-β. Finally, the ability of LAP, the type II ED, the type II ED/Fc chimera, and the type III ED to antagonize binding of TGF-β to its cell-surface receptors was tested using mink lung epithelial cells stably transfected with a TGF-β-sensitive luciferase reporter gene. These results were correlated with the BIAcore data.

The present analyses strongly support the existence of a complex consisting of one type III receptor, two TGF-β molecules, and four type II receptors within the signaling receptor complex. Additionally, results of our kinetic analysis indicate that the apparent dissociation rate constant may be the best predictor of the antagonistic potency of TGF-β-binding proteins.

**EXPERIMENTAL PROCEDURES**

**Equipment and Reagents**

The BIAcore 1000, CMS sensor chips, N-hydroxysuccinimide, N-ethyl-N’-(3-diethylaminopropyl)carbodiimide hydrochloride, and 1 M ethanolamine (pH 8.5) were purchased from BIAcore Inc. (Piscataway, NJ). Recombinant TGF-β isoforms (human recombinant TGF-β1, TGF-β2, and TGF-β3) expressed in Chinese hamster ovary, NSO, and ST21 cells, respectively, recombinant β1-LAP (expressed in Chinese hamster ovary cells), and the recombinant extracellular domains of the TGF-β type II and III receptors in addition to the TGF-β type II receptor/Fc chimera corresponding to the 159 amino acid residues of the type II ED fused to the Fc region of human IgG1 (all expressed in NSO cells) were provided by R&D Systems (Minneapolis, MN).

**Surface Plasmon Resonance Experiments**

Immobilization of TGF-β on Sensor Chips—TGF-β isoforms were coupled to CMS sensor chip surfaces using the standard amine coupling procedure and a flow rate set at 5 μl/min. Sequential injections consisted of a 0.05 M N-hydroxysuccinimide and 0.2 M N-ethyl-N’-(3-diethylaminopropyl)carbodiimide hydrochloride mixture (35 μl) followed by TGF-β solutions (0.8–1.6 μg/ml) in 10 mM acetic acid (pH 4.0) until the desired amount of coupled TGF-β was reached. A solution of 0.1 M ethanolamine HCl (35 μl, pH 8.5) was then used to block the remaining activated carboxyl groups. Control dye-free surfaces were also generated by replacing the TGF-β solutions with running buffer (20 mM Hepes (pH 7.4), 150 mM NaCl, 3.4 mM EDTA, and 0.05% Tween 20).

Kinetic Assays on the BIAcore—All the kinetic experiments were carried out at 25°C at a flow rate of 5 μl/min, except for the mass transport experiments, for which different injections of each analyte (human LAP, the type II ED, the type II ED/Fc chimera, and the type III ED) were performed at flow rates ranging from 5 to 20 μl/min. Running buffer was used for diluting all the species that were injected over the TGF-β isofrom surfaces. Different concentrations of LAP (73 μl), the type II ED (27 kDa), the type II ED/Fc chimera (120 kDa), and the type III ED (112.5 kDa) were injected over various TGF-β (25 kDa) surfaces as well as over a control surface for 300 s, following which the analyte solutions were replaced by buffer for 300 s. Regeneration of the sensor chip for subsequent injections was accomplished by two pulses of 0.1 M ethanolamine HCl (20 mM, 120 s), followed by an EXTRACLEAN procedure done according to the BIAcore manual.

Data Preparation and Analysis—Sensorgrams were prepared and globally fitted using nonlinear least-squares analysis and numerical integration of the differential rate equations using the SPRRevolution software package. The data preparation was done mainly as described elsewhere (19). Briefly, each sensorgram generated using a control surface was subtracted from the corresponding experimental sensorgrams, and the resulting curves were transformed to concentration units using the molecular mass of the injected species, the equivalence of 1000 resonance units (RU) per 1 ng/mm², and a matrix thickness of 100 nm. Each data set (which consists of sensorgrams from injections of different analyte concentrations over the same surface) was then analyzed using several different kinetic models that are available in the SPRRevolution software.

Mathematical Modeling and Parameter Estimation—In SPRRevolution, the various kinetic models were transposed into differential equations by making a mass balance on all species present in the dextran matrix. These sets of nonlinear differential equations were solved numerically using an adaptive step-size Runge-Kutta method (20). The resulting routine was used in a nonlinear regression program using Marquardt’s algorithm (21) to estimate the values of the constants from experimental data. The schematic representation of the models used for the data analysis and their related sets of differential rate equations are shown in Table 1. For each set of kinetic parameters, we used the active quantity of ligand immobilized on the matrix were considered as global parameters for a given set of curves. Moreover, two local parameters were added for each curve to take into account the refractive index changes at the beginning of the wash-on and wash-off phases.

**Evaluation of the Quality of the Fit for the Various Kinetic Models—** For each set of residuals, the statistical values were calculated. The first statistical value is the S.D. of the residuals. The second statistical value is the "+ or -" sign statistic (Z+2). Each residual is replaced by its sign value (+ or -), and the following statistic is then calculated on the newly created data set, Z+2 = (n × (R1 - 1) - 2 × n1 × n2/2(1/2)) × n1 × n2/2(n1 × n2 - n(n1 + 1))/2, where R1 is the number of positive runs, n1 is the + number, and n2 is the - number. The third statistical value was run up and down (Z+2) (22). Using the residual set xi(t), a new set of data, yi, is created with yi(t) = xi(t) - x(t + 1). As for the above test, each y value is then replaced by its sign value (+ or -). If we call R2 the number of positive yi(t)'s of the runs, then the statistic Z2 equals (-(Z+2 - (2 × n - 1)/3))1/2(16 × n - 29)/40. Assuming that the residuals are independent, the Z1 and Z2 statistics follow a normal law with a mean equal to 0 and a variance equal to 1.

**BIAcore Multiple Binding Experiments—**The effect of pre-binding of LAP was assayed as follows. 300-s injections of LAP solutions (ranging in concentration from 0 to 200 nM in the case of TGF-β1 or from 0 to 400 nM for TGF-β2 and TGF-β3) were performed over TGF-β isofrom surfaces (155, 250, and 250 RU of coupled TGF-β1, TGF-β2, and TGF-β3, respectively) and a control surface. All injections were followed by a short wash phase (100 s) and a 300-s injection of the type II or III ED at a fixed concentration of 400 or 600 nm, respectively. The quantity of each ectodomain bound after 300 s was expressed as a percentage of binding after 300 s in the absence of LAP. This was plotted against the amount of LAP pre-bound to the surface, which was expressed as the percentage of maximum LAP binding and was determined by a numerical rearrangement approach.

The effect of pre-binding of the type II and III EDs was assayed as follows. Injections of the type III ED (0–800 nM, 300 s) were followed by 300-s co-injections of a mixture of the type II ED (at the same concentration as for the first injection) and the type II ED at 500 nm. The reverse experiment was also carried out. The results were expressed as percent binding of the ectodomain that was injected second, and this was plotted against the concentration of the ectodomain injected first.

**Testing the Antagonistic Inhibitor Potency of the Type II and III EDs and LAP Using a Luciferase Assay in Mink Lung Epithelial Cells**

Mink lung epithelial cells stably transfected with the PAI-1 promoter fused to the firefly luciferase reporter gene were a generous gift of Dr. D. Rifkin (23). These cells were plated in 96-well tissue culture plates (2 × 10³ cells/well) in Dulbecco’s modified Eagle medium containing 5% fetal bovine serum and were allowed to attach for at least 3 h at 37°C in a 5% CO₂ atmosphere. Cells were then lysed and assayed for luciferase activity using the Promega luciferase assay kit according to the manufacturer’s instructions. Luminescence was measured with a Berthold Lumat LB9501 luminometer. The activity was expressed as the percentage of the activity of each TGF-β isoform in the absence of LAP, the type II ED, the type II ED/Fc chimera, or the type III ED.

**RESULTS**

**BIAcore Analysis of TGF-β Interactions with LAP, the Type II ED, the Type II ED/Fc Chimera, and the Type III ED—**The first goal of our study was to determine the kinetic mechanisms and kinetic constants related to the interactions of three mammalian TGF-β isoforms with four different proteins: TGF-β1 LAP and type II and III EDs, in addition to an artificially dimerized type II ED (the type II ED/Fc chimera). In a typical BIAcore experiment, one of the binding partners is immobilized
on the sensor chip surface (called the ligand in BIAcore terminology). A solution containing the other binding partner is injected over the sensor chip surface (called the analyte in BIAcore terminology). The mass accumulation of the analyte on the surface as it binds to the ligand is recorded in arbitrary resonance units, which vary with the refractive index of the interfacing medium. As the change in the refractive index is a linear function of the surface concentration of the macromolecules, the RU signal is proportional to the mass accumulation of the analyte. This constitutes the wash-on phase of the experiment. After a certain period of time, the analyte solution is replaced by buffer, and the dissociation of surface complexes is monitored (the wash-off phase). Since the wash-on and wash-off phases of the curve (sensorgram) are recorded in real time, it is possible to derive kinetic parameters from these sensorgrams.

In preliminary experiments, injection of TGF-β1 over a control dextran surface (no ligand immobilized) resulted in a significant increase in the surface plasmon resonance signal with time, indicating that TGF-β1 binds nonspecifically to the dextran surface. In contrast, no interaction was detectable when LAP, the type II ED, the type II ED/Fc chimera, and the type III ED were injected over the control surface (data not shown). Therefore, we immobilized the TGF-β isoforms on the sensor chip and injected LAP, the type II ED, the type II ED/Fc chimera, or the type III ED over the surface.

In preliminary experiments using TGF-β-coupled surfaces with LAP, type II ED, type II ED/Fc chimera, or type III ED flowing, the binding data were fit using nonlinear least-squares analysis and numerical integration of the differential rate equations corresponding to a simple kinetic model. This model did not yield a good fit as judged by the non-random distribution in the residuals (the difference between calculated and experimental data) (data not shown). This deviation from a simple one-to-one interaction model may result from the existence of a more complex biological interaction. Alternatively, it may be due to the presence of artifacts such as mass transport and re-binding effects or steric hindrance/crowding problems (24) that can occur when the BIAcore experimental conditions are not optimized. To minimize these artifacts and to perform kinetic studies, we generated different surfaces where low quantities of TGF-β isoforms were coupled (from 50 to 700 RU). This approach reduces so-called crowding problems by minimizing the masking of potential ligand-binding sites on the surface by accumulated ligand-analyte complexes. Additionally, it reduces mass transport and re-binding artifacts that occur if the transportation rate of the analyte through the unsterred layer over the surface is slow compared with the kinetics of binding.

Based on the results from these preliminary experiments, we coupled the minimum amount of each TGF-β isoform required to obtain an acceptable signal-to-noise ratio when injecting LAP, the type II ED, the type II ED/Fc chimera, or the type III ED. Due to the different apparent kinetics and affinities of these binding proteins for TGF-β, it was necessary to use surfaces with different amounts of coupled TGF-β for each interaction. For each TGF-β isoform surface, the absence of mass transport limitation and/or re-binding artifacts was tested by injecting the lowest concentration of the analyte used in the kinetic study at different flow rates ranging from 5 to 30 μl/min. Since the sensorgrams were superimposable when varying the flow (data not shown), it can be concluded that the diffusion of the analyte from the bulk flow to the matrix is not kinetically limiting under these flow and surface conditions.

We next injected LAP, type II ED, type II ED/Fc chimera, and type III ED solutions ranging in concentration from 12.5 to 200, 32 to 355, 18 to 500, and 50 to 800 nM, respectively, over the TGF-β1, TGF-β2, and TGF-β3 surfaces that were optimized for each binding partner. The sensorgrams corresponding to the experiments using TGF-β3 surfaces are shown in Figs. 1–4. Similar sensorgrams were observed for the TGF-β1 and TGF-β2 surfaces (data not shown). Each set of sensorgrams was analyzed by curve fitting using the SPRevolution® software package (19, 25). This method of data analysis has been shown to be critical for distinguishing between different binding models and determining kinetic constants that are consistent with all the time points in all the sensorgrams in one set (26–28). In all cases, we observed a poor fit when using a simple one-to-one model to depict the interactions (Figs. 1–4, panels C, and data not shown). Since artifacts such as mass transport limitation and re-binding and crowding problems were already eliminated or minimized, we tested if such deviations from fitting a simple model could be due to more complex interaction mechanisms.

We tested three more complex kinetic models for each TGF-β-binding protein/TGF-β data set. The selection of these more complex models was based on their potential biological relevance (see “Discussion” for literature references to these mod-

![Fig. 1. Global analysis of the LAP interaction with the TGF-β3 isoform. A, global fit of the LAP/TGF-β3 interaction sensorgrams. Different concentrations of LAP ranging from 12.5 to 200 nM were injected over 150 RU of immobilized TGF-β3 and over a control dextran surface. The points are the resonance units obtained after blank subtraction, and the solid lines represent the fit when integrating all the curves simultaneously using a rearrangement model. B, residuals from the fit of the LAP/TGF-β3 interaction using the rearrangement model. C, residuals from the fit of the LAP/TGF-β3 interaction using a simple one-to-one model.](http://www.jbc.org/content/285/19/29634/F1.large.jpg)
els). These models included 1) a model depicting the occurrence of a rearrangement in the TGF-β-binding protein-TGF-β complex; 2) a model assuming the presence of two independent binding sites on the TGF-β molecule (two-sited ligand model); and 3) a model depicting the presence of two distinct binding sites on the injected binding protein (this can also be termed an avidity model since it involves the binding of a bivalent analyte to a fixed ligand). The schematic representations of the models and their respective differential rate equations are shown in Table I. The residual plots, the S.D. of the residuals, as well as the $Z_1$ and $Z_2$ statistics can be used to judge the adequacy of the models when tested against the same data set. That is, when a kinetic model adequately depicts a biomolecular interaction, the residuals will be minimal and randomly distributed around a zero value. More specifically, a significantly large amplitude of the residuals, long series of positive or negative points, or long series of ascending or descending points will be absent from the residual plot. These characteristics will be reflected in low values for the S.D., the $Z_1$ statistic, and the $Z_2$ statistic, respectively.

Based on this type of analysis, it was observed that the LAP interaction with TGF-β3 was best fit by a rearrangement model (Fig. 1, A and B; and Table II). The same conclusion was made for TGF-β1 and TGF-β2 binding to LAP (data not shown). An analysis of the type II ED injections over TGF-β3 indicated that this interaction was best fit by the two-sited ligand model (Fig. 2, A and B; and Table II). The same model was determined to be the most adequate for the type II ED/TGF-β1 interaction (data not shown). No interaction was detected between the TGF-β2 isoform and the type II ED, which is consistent with our previous results (29). A model assuming the presence of two binding sites on the type III ED (avidity model) gave the best fit for the TGF-β2 isoform (Fig. 3, A and B; and Table II) and the TGF-β1 isoform (data not shown). In the case of the TGF-β2 isoform interacting with the type III ED, the fit was relatively good, but essentially the same for the rearrangement and the two-sited analyte (avidity) models (data not shown). Finally, in the case of the type II ED/Fc chimera, the TGF-β3 isoform interaction was best fit by a rearrangement model (Fig. 4, A and B; and Table II). The same model was found to be the best for the TGF-β1 isoform.

To confirm the stoichiometries of binding that were determined above using the different surfaces that were optimized for each binding partner/TGF-β isoform interaction and to provide an additional test for internal self-consistency, we next injected the LAP and type II and III ED binding proteins over the same TGF-β1 surface (125 RU immobilized). Once again, the rearrangement, two-sited ligand, and avidity models best described the interaction of TGF-β1 with LAP and the type II and III EDs, respectively (Fig. 5, A–C, respectively). The same comparison could not be carried out on a TGF-β2 surface since the type II ED does not detectably bind TGF-β2. Also, a TGF-β3 surface could not be used for a similar experiment due to the differences in the apparent affinities of the three binding proteins for this isoform (Table II). The best fitting kinetic model is indicated in boldface type. These results are representative of at least three experiments.
ligand, and two-sited binding protein models, when applied to the LAP and type II and III ED interactions, respectively, yielded similar values for the amount of active TGF-β1 on the surface (ranging from 27 to 33 RU). No other combination of models gave a consistent value for the amount of active TGF-β1 on the surface. This further supports the validity of the different kinetic models. Additionally, the kinetic constants determined in this experiment were similar to those determined using the optimized TGF-β1 isoform surfaces (10% difference) (data not shown).

The stoichiometry of binding for the type II ED/Fc chimera was confirmed by injecting the type II ED/Fc chimera and the type II ED over the same TGF-β1 and TGF-β3 surfaces. The amounts of immobilized active TGFs-β were first calculated by curve fitting the type II ED/Fc chimera sensorgrams using the 1:1 stoichiometry rearrangement model. These numbers were then compared with the amounts of immobilized active TGFs-β determined by saturating the surfaces with the type II ED (injections of 1.5 and 2 μM type II ED solutions were used to confirm that the surfaces were saturated). In all cases, we obtained the same value for the amount of active TGFs-β within a 1-RU difference only when assuming a 1:1 stoichiometry for the type II ED/Fc chimera and a 2:1 stoichiometry for the non-dimerized type II ED/TGF-β3 interaction.

The thermodynamic and kinetic parameters for each TGF-β-binding protein/TGF-β isoform interaction, calculated using the relevant kinetic model and data from optimized surfaces (Figs. 1–4 for TGF-β3 and data not shown for TGF-β1 and TGF-β2), are presented in Tables IV–VII.

**Analysis of the Ability of LAP and the Type II and III EDs to Block Subsequent Binding of the Type II or III ED**—To use the BIAcore to determine if these three binding proteins interact with TGF-β1 in independent, competitive, or cooperative manners, we investigated the effect of pre-binding these three proteins to immobilized TGF-β1 on the subsequent binding of the type II and III receptor ectodomains. The influence of LAP binding on the subsequent binding of the type II and III EDs to TGF-β1 was relatively straightforward to investigate since LAP has a slow dissociation rate (see Fig. 1A). In other words, enough LAP will remain bound during a subsequent injection so that its effect on type II or III ED binding will be detectable. Specifically, injections of LAP at different concentrations were followed by a short buffer injection and then an injection of the type II or III ED at a fixed concentration (400 and 600 nM, respectively). For TGF-β1 and TGF-β3 isoform surfaces, type II
ED binding was reduced in a dose-dependent manner by pre-binding increasing amounts of LAP (Fig. 6A and B). Since the type II ED does not detectably bind to TGF-β2, this experiment was not done on that isoform surface. Comparable results were obtained when LAP was bound to the three TGF-β isoforms prior to the binding of the type III ED (Fig. 6C). That is, LAP inhibited subsequent binding of the type III ED to all TGF-β isoforms in a dose-dependent manner.

Since the dissociation of the type III ED from the TGF-β isoform surfaces is faster than the dissociation of LAP (compare Figs. 1 and 3), we could not use exactly the same experimental strategy to examine its influence on subsequent type II ED binding events. A different type of experiment was performed in which type III ED injections (at various concentrations) were followed immediately by injections containing the type III ED (at the same concentration as in the first injection) together with the type II ED at a fixed concentration. In this way, the dynamics of binding and dissociation of TGF-β with the type III ED were maintained, and it was possible to monitor any perturbation of type II ED binding to TGF-β1 or TGF-β3 caused by the presence of the type III ED. As shown in Fig. 6D, both ectodomains could bind simultaneously to TGF-β, and the binding of one did not hinder the binding of the other. The same experiment was conducted over the TGF-β2 surface. Due to the ability of the type III receptor to enhance TGF-β2 binding to the type II receptor at the cell surface (9, 10), it might be expected that the type II and III EDs would exhibit cooperative binding to TGF-β2 on the BIACore. In the presence of the type III ED, the type II ED still did not exhibit any detectable binding to TGF-β2 (Fig. 6E). Reverse experiments examining the influence of type II ED pre-binding on the subsequent binding of the type III ED to TGF-β1 and TGF-β3 were also carried out. As expected from the previous set of experiments, type II ED binding neither blocked nor enhanced subsequent type III ED binding (data not shown).

Ability of LAP, the Type II ED, the Type II ED/Fc chimera, and the Type III ED to Antagonize TGF-β Receptor Signaling—The ability of LAP, the type II ED, the type II ED/Fc chimera, and the type III ED to block TGF-β interactions with cell-surface receptors and subsequent signaling was tested with mink lung epithelial cells stably transfected with a luciferase reporter gene. As shown in Fig. 7A, LAP was able to inhibit TGF-β-induced receptor signaling for the three TGF-β isoforms. LAP inhibited receptor signaling by 50% at a 10-fold lower concentration for TGF-β3 as compared with TGF-β1 and TGF-β2 (the IC_{50} was ~1 nM for TGF-β3 versus 10 nM for TGF-β1 and TGF-β2). Similar to LAP, the type III ED also exerted an inhibitory effect on TGF-β-induced signaling for all three TGF-β isoforms (Fig. 7B). However, the IC_{50} for the type III ED inhibitory effect was found to be ~5 nM for the TGF-β2 and TGF-β3 isoforms, whereas an IC_{50} of ~50 nM was observed in the case of the TGF-β1 isoform. No significant blocking activity was observed with the type II ED up to a concentration of 500 nM (Fig. 7C) for all three TGF-β isoforms. In contrast, the artificially dimerized type II ED/Fc chimera exhibited an inhibitory effect with an IC_{50} of ~100 pM for the TGF-β1 and TGF-β3 isoforms (Fig. 7D).

**DISCUSSION**

To study the interaction between TGF-β and four binding proteins (LAP, type II ED, type II ED/Fc chimera and type III ED) using the BIACore, we immobilized three TGF-β isoforms individually on sensor chips and injected the four binding proteins over the surfaces. This immobilization strategy was chosen since injected TGF-β interacted nonspecifically with a control surface. Our preliminary kinetic analysis revealed that, as observed for many systems studied with the BIACore, these binding protein/TGF-β interactions failed to be represented as a simple one-to-one interaction. This type of deviation from pseudo first-order kinetics may be explained by artifacts related to less than optimal experimental design (19, 24, 27). On the other hand, these deviations may be explained by the occurrence of more complex interaction mechanisms. After optimizing the experimental design and still observing deviations from a simple kinetic model for the four binding proteins, we tested more complex interaction models by globally fitting the data (i.e., all the curves are fit at the same time). This technique has been shown to be the best for discriminating between different complex binding mechanisms (26, 28) and for determining the related kinetic constants (27).

For all three TGF-β isoforms, the LAP binding data were best fit by a model depicting a one-to-one interaction, followed by a rearrangement of the LAP-TGF-β complex (Fig. 1). Type II ED interactions with TGF-β1 and TGF-β3 isoforms were best described by a model assuming that TGF-β possesses two independent binding sites for the type II receptor (Fig. 2). Consistent with a preliminary study performed in our laboratory (29), we could not detect any interaction between the type II ED and the TGF-β2 isoform, even when high amounts of TGF-β2 were immobilized. The type III ED interaction data were best fit with an avidity model, which depicts the presence of two independent binding sites on the type III ED, with these sites being able to bind sequentially to two immobilized TGF-β molecules (Fig. 3). Finally, the type II ED/Fc chimera binding data were best fit, as with LAP, by the 1:1 stoichiometry rearrangement model (Fig. 4). These different stoichiometries of binding...
and kinetic models were confirmed by carrying out internal consistency tests in which these TGF-β-binding proteins were injected over the same TGF-β surfaces (Fig. 5 and Table III).

The LAP interaction with the TGF-β1 isoform has previously been studied on the BIAcore by Bailly et al. (30). They were able to fit the sensorgrams with a simple one-to-one model by analyzing the data using the integrated rate equation method. Global analysis of the data was not performed since the software tools for this type of analysis were not generally available at that time. Our results are in agreement with theirs in that our best fitting model depicts a 1:1 stoichiometry for the LAP/TGF-β interaction not only for TGF-β1, but also for TGF-β2 and TGF-β3. However, the global fit of the data revealed a second step involving a rearrangement of the complex. The 1:1 stoichiometry for the LAP/TGF-β interaction is not unexpected since LAP is the remnant of the precursor form of TGF-β. Accordingly, during biosynthesis, LAP and TGF-β would be associated as a one-to-one complex. The finding that this complex undergoes a rearrangement can be interpreted in two ways. Since both TGF-β and LAP are covalent dimers, it can be proposed that the first step in complex formation involves the interaction between one monomer of each of LAP and TGF-β and that the rearranged complex corresponds to a complex in which both monomers of LAP and TGF-β are interacting. This second binding step would have zero order kinetics since it occurs intramolecularly. Alternatively, the rearrangement step may correspond to a conformational change in the complex. This latter mechanism is supported by far-UV circular dichroism studies performed by McMahon et al. (31), who showed that the LAP/TGF-β1 complex undergoes a major structural rearrangement after binding. We also recently found that BIAcore data for the interaction of TGF-α with the epidermal growth factor receptor extracellular domain could be fit with a conformational change model (28). In this case, the model was also supported by far-UV circular dichroism studies (32). A comparison of the apparent thermodynamic $K_d$ values (defined as $K_d(app) = ([LAP] \times [TGF-β]) / ([LAP] + [TGF-β])$) (Table IV) for the LAP interaction with the three TGF-β isoforms reveals that the affinity of LAP for each TGF-β isoform is very similar and in the low nanomolar range. This is in close agreement with the value estimated by Bailly et al. (30) for the LAP/TGF-β1 interaction using a simple model. This relatively high affinity for the LAP/TGF-β interaction results primarily from a relatively slow $k_{diss}$ dissociation rate ($3-5 \times 10^{-4}$ s$^{-1}$) (Fig. 1 and Table IV).

In the case of the type II ED interaction with TGF-β1 and TGF-β3, the finding that the best fitting model involves a stoichiometry of two receptors for one ligand is consistent with the dimeric and symmetrical nature of the TGF-β molecule (33–35). Moreover, Matsuzaki et al. (36) also reported the formation of a type II receptor homodimer that complexes one TGF-β1 molecule by expressing full-length recombinant receptors in insect cells. Additionally, it has been shown in in vitro experiments that the stoichiometry of binding of the type II ED to the TGF-β3 isoform is 2:1 (37). Somewhat surprisingly, in light of the symmetrical nature of the TGF-β structure, we found that the thermodynamic $K_d$ values determined for each type II ED-binding site within the TGF-β dimer differ by 5–7-fold, depending on the TGF-β isoform (Table V). This affinity difference may be the result of the coupling procedure we used to immobilize TGF-β. That is, amine coupling may have gener-

**TABLE III**

Quantity of active immobilized TGF-β1 as determined by numerical analysis of the LAP, type II ED, and type III ED/TGF-β1 senso

| Kinetic model used                  | LAP  | Type II ED | Type III ED |
|------------------------------------|------|------------|-------------|
| Rearrangement of complex           | 28.3 ± 0.5 | 43.0 ± 1.2 | 15.4 ± 0.4   |
| Two sites on ligand                | 21.5 ± 3.5 | 33.0 ± 1.0 | 7.5 ± 0.2    |
| Two sites on analyte (avidity)     | 65.3 ± 3.6 | 13.1 ± 1.5 | 27.0 ± 0.8   |

**FIG. 5.** Global analysis of the LAP and type II and III ED interactions with the TGF-β1 isoform on the same surface. **A:** upper panel, different concentrations of LAP ranging from 25 to 400 nM were injected over 125 RU of immobilized TGF-β1 and over a control dextran surface. The points are the resonance units obtained after blank subtraction, and the solid lines represent the fit when integrating all the curves simultaneously using a rearrangement model. **Lower panel,** shown are the related residuals. **B:** upper panel, different concentrations of the type II ED ranging from 25 to 400 nM were injected over the same surfaces as described for **A.** The points are the resonance units obtained after blank subtraction, and the solid lines represent the fit when integrating all the curves simultaneously using a two-sited ligand model. **Lower panel,** shown are the related residuals. **C:** upper panel, different concentrations of the type III ED ranging from 37.5 to 600 nM were injected over the same surfaces as described for **A.** The points are the resonance units obtained after blank subtraction, and the solid lines represent the fit when integrating all the curves simultaneously using a two-sited analyte (avidity) model. **Lower panel,** shown are the related residuals.
Table IV
Kinetic and thermodynamic constants for TGF-β isoforms interacting with LAP

| Kinetic parameters | TGF-β1 isoform | TGF-β2 isoform | TGF-β3 isoform |
|--------------------|----------------|----------------|----------------|
| $k_{a1}$ (M$^{-1}$ s$^{-1}$) | (1.7 ± 0.3) × 10$^4$ | (4.3 ± 0.2) × 10$^4$ | (1.22 ± 0.04) × 10$^5$ |
| $k_{d1}$ (s$^{-1}$) | (3.4 ± 1.5) × 10$^{-2}$ | (2.4 ± 0.2) × 10$^{-2}$ | (1.9 ± 0.1) × 10$^{-2}$ |
| $k_{d2}$ (s$^{-1}$) | (4.9 ± 0.9) × 10$^{-2}$ | (1.55 ± 0.06) × 10$^{-2}$ | (1.34 ± 0.06) × 10$^{-2}$ |
| $K_{a1}$ (nM)$^a$ | 1900 ± 1200 | 560 ± 70 | 150 ± 100 |
| $K_{d2}$ (no unit)$^b$ | (1.0 ± 0.4) × 10$^{-2}$ | (1.5 ± 0.2) × 10$^{-2}$ | (2.1 ± 0.3) × 10$^{-4}$ |
| $K_{d2}$ (nm)$^b$ | 23.5 ± 3.5 (n = 2) | 6.8 ± 2.5 (n = 5) | 3.5 ± 1.1 (n = 3) |

$^a$ $K_{d1}$ corresponds to the thermodynamic constant related to the first binding step (see Table I for the description of the model).
$^b$ $K$ is defined as $K_{d(app)} = 1/K_{d(app)}$ where $K_{d(app)} = K_a × (1 + K_{a2})$ with $K_{a2} = k_{d2}/k_{a1}$ and $K_{a1} = k_{a1}/k_{d1}$. $K_{d(app)}$ corresponds to the global thermodynamic constant related to the interaction since $K_{d(app)} = ([L] × [A]/([LA] + [LA^*]}})). The values given for $K_{d(app)}$ correspond to the mean ± S.D. of $n$ independent experiments.

![Fig. 6](http://www.jbc.org/)

**Fig. 6.** Analysis of the ability of LAP and the type II and III EDs to block the subsequent binding of type II or III ED. A, example of the effect of LAP pre-binding on the subsequent binding of the type II ED to TGF-β3. B, effect of LAP pre-binding on the subsequent type II ED binding to the TGF-β1 and TGF-β3 isoforms. Results from A and similar experiments conducted with the TGF-β1 isoform were analyzed and plotted as described under “Experimental Procedures.” The y axis corresponds to the amount of type II ED bound at the end of the type II ED injection and is expressed as a percentage of type II ED binding in the absence of LAP pre-binding. The x axis corresponds to the amount of LAP that was pre-bound at the end of the LAP injection and is expressed as a percentage of maximum LAP binding for that surface. Maximum LAP binding was determined by integrating numerically the sensorgrams using a rearrangement model. C, effect of LAP pre-binding on subsequent type III ED binding to the three TGF-β isoforms. Similar experiments were conducted with the type III ED and plotted as with the type II ED (see “Experimental Procedures”). D, effect of type III ED on type II ED binding to the TGF-β1 and TGF-β3 isoforms. Injectations of the type III ED (0–800 nM) were immediately followed by co-injection of a mixture of the type III ED (at the same concentration) and the type II ED at 500 nM. The results are expressed as a percentage of type II ED binding in the absence of the type III ED and are plotted against the concentration of type III ED injected. E, effect of type III ED binding on type II ED binding to the TGF-β2 isoform. Injection of the type III ED (800 nM) followed by injection of a type III ED (800 nM)/type II ED (400 nM) mixture over a TGF-β2 surface gave a sensorgram that was similar to the one corresponding to the injection of the type III ED (800 nM) alone.

ated asymmetry in the immobilized TGF-β, thereby reducing the affinity of one of the two sites. Even though amine coupling may have somewhat affected the affinity of one site within the TGF-β dimer, it is unlikely that the stoichiometry of TGF-β1 and TGF-β3 binding to type II ED has been perturbed. With this proposed immobilization effect in mind, it can be concluded that the equilibrium $K_a$ for the high affinity site is likely to be the most relevant. This $K_a$ was determined to be in the 100 nM range for both the TGF-β1 and TGF-β3 isoforms, and it results from $k_d$ values of $-6 \times 10^{-5}$ M$^{-1}$ s$^{-1}$ and $k_d$ values of $-6 \times 10^{-2}$ s$^{-1}$ (Table V).

In the case of the type II ED/Fc chimera, the model found to best depict the interaction was a one-to-one interaction, followed by a rearrangement of the complex. The apparent $K_d$ values were found to be equal to 60 and 50 nM for TGF-β1 and TGF-β3, respectively (see Table VII). The change in the stoichiometry between the type II ED/Fc chimera model and the type II ED model is not surprising in that the artificial dimer-
Kinetic and thermodynamic constants for TGF-β isoforms interacting with the type II ED

| Kinetic parameters | Kinetic model: two sites on TGF-β |
|--------------------|----------------------------------|
|                    | TGF-β/II isoform                  | TGF-β/III isoform                   |
| $k_{a1}$ (M$^{-1}$ s$^{-1}$) | $(5.4 \pm 0.3) \times 10^6$       | $(6.1 \pm 0.1) \times 10^6$         |
| $k_{a2}$ (M$^{-1}$ s$^{-1}$) | $(6.5 \pm 0.2) \times 10^7$       | $(4.8 \pm 0.07) \times 10^7$        |
| $k_{d1}$ (M$^{-1}$ s$^{-1}$) | $(1.8 \pm 0.1) \times 10^5$       | $(2.5 \pm 0.1) \times 10^5$         |
| $K_{d1}$ (nM)       | $158 \pm 37$ (n = 6)              | $150 \pm 100$ (n = 3)               |
| $K_{d2}$ (nM)       | $981 \pm 123$ (n = 6)             | $294 \pm 166$ (n = 3)               |

Note: All the notations for the constants used below are consistent with the ones used in Table I. The values given for $K_{d1}$ and $K_{d2}$ correspond to the mean ± S.D. of $n$ independent experiments.

Table V

FIG. 7. Evaluation of the antagonistic potency of LAP, the type II and III EDs, and the type II ED/Fc chimera using a TGF-β-responsive luciferase reporter assay in mink lung epithelial cells.

The cells were incubated with TGF-β isoforms in the absence or presence of varying concentrations of LAP (A), the type III ED (B), the type II ED (C), and the type II ED/Fc chimera (D), and the luciferase activity was assayed as described under “Experimental Procedures.”

Independent TGF-β-binding sites on the type III ED (Tables II and III). Despite the good quality of the fit when using this two-sited receptor model (avidity), the calculated kinetic and equilibrium constants (Table VI) must be considered with great care, as emphasized by Muller et al. (39). They showed that kinetic constants that are derived with an avidity model are difficult to interpret in meaningful terms, even in the simpler case of a bivalent antibody where the two sites are equal. However, they also concluded that the avidity model is still relevant for distinguishing among different kinetic models. Since the constants determined using the avidity model and global fitting (Table VI) must be interpreted with caution, we also estimated the apparent thermodynamic $K_d$ values for the type III ED/TGF-β isoform interactions using the plateau values from the sensorgrams in Scatchard plots (data not shown). These calculations estimated the equilibrium $K_d$ values to range between 300 and 1000 nM. It must be emphasized that these numbers are approximate since the plateaus were only being approached in some of the sensorgrams.

To determine if LAP and the type II and III EDs bind to TGF-β in independent, competitive, or cooperative manners, we studied, using the BIAcore, if the binding of one of these three proteins to TGF-β would alter the subsequent binding of the type II or III ED. When varying concentrations of LAP were injected prior to the type II or III ED, we observed a dose-dependent decrease in the binding of both ectodomains (Fig. 6, A–C). These results indicate that LAP binding to TGF-β inhibits its binding to three receptor sites: one on the type II ED and two on the type III ED. The conformational change model for the LAP/TGF-β interaction may provide a simple explanation for this result if one assumes that the conformational change occurs in the TGF-β moiety within the complex. In other words, a conformational change could readily affect several binding sites within the TGF-β ligand. We cannot exclude, however,
that LAP binds to TGF-β in such a way that it sterically blocks binding to the type II ED and both sites on the type III ED.

In contrast, in these BLACore multiple binding experiments, type II and III EDs were found to bind to TGF-β1 and TGF-β3 in an independent fashion since the pre-binding of one did not affect the subsequent binding of the other (Fig. 6D and data not shown). This indicates that the binding sites on TGF-β(g) for these two extracellular domains are distinct and non-cooperative. Additionally, type III ED pre-binding to TGF-β2 was not able to promote type II ED binding to the TGF-β2 isofrom (Fig. 6E), as might have been expected from the observation that the type III receptor is able to enhance TGF-β2 binding to the type II receptor at the cell surface (9, 10). These results suggest that the extracellular domains of the type II and III receptors are not sufficient to mimic the type III receptor-enhanced binding of TGF-β2 to the type II receptor and that the transmembrane and/or cytoplasmic domains must be involved in this process. This conclusion is supported by our data showing that the cytoplasmic domains of the type II and III receptors interact such that an antibody epitope on the type III receptor cytoplasmic domain is masked by the presence of the type II receptor (41).

These BLACore experiments were complemented by a luciferase reporter assay in mink lung epithelial cells, which was used to evaluate the antagonistic potency of LAP, the type II ED and Fe chimera, and the type III ED (Fig. 7). LAP was found to inhibit TGF-β signaling for all three TGF-β isoforms with IC₅₀ values of ~1 nM for TGF-β3 and ~10 nM for TGF-β1 and TGF-β2. Similar to LAP, the type III ED also inhibited signaling for all three TGF-β isoforms. However, the IC₅₀ values were ~50 nM for TGF-β1 and ~5 nM for TGF-β2 and TGF-β3. No inhibition of signaling was observed at any type II ED concentration up to 500 nM. In contrast, the type II ED/Fe chimera was found to be the best antagonist for TGF-β1 and TGF-β2 with an IC₅₀ of 100 pm for both isoforms. Our results on the neutralizing potency of the type II ED and the type II ED/Fe chimera are similar to those of Komel et al. (40) for TGF-β1.

**Table VI**

| Kinetic parameters | TGF-β1 isofrom | TGF-β2 isofrom | TGF-β3 isofrom |
|--------------------|----------------|----------------|----------------|
| kₑᵥ (M⁻¹ s⁻¹)     | (1.4 ± 0.4) × 10⁴ | (1.6 ± 0.4) × 10⁴ | (1.7 ± 0.2) × 10⁴ |
| kₑᵥ (s⁻¹)         | (2.9 ± 0.1) × 10⁻² | (4.0 ± 0.2) × 10⁻² | (4.0 ± 0.2) × 10⁻² |
| kₑᵥ (M⁻¹ s⁻¹)     | (6.8 ± 0.4) × 10² | (1.7 ± 0.7) × 10² | (0.7 ± 0.1) × 10³ |
| kₑᵥ (s⁻¹)         | (3.5 ± 0.2) × 10⁻³ | (2.6 ± 0.6) × 10⁻³ | (2.2 ± 0.3) × 10⁻³ |
| Kₑᵥ (nM)          | 2074 ± 560 (n = 3) | 2500 ± 50 (n = 3) | 4560 ± 1500 (n = 3) |
| Kₑᵥ (nM)          | 4274 ± 1340 (n = 3) | 2173 ± 800 (n = 3) | 3990 ± 1200 (n = 3) |

- *kₑᵥ* corresponds to the thermodynamic constant related to the first binding step (see Table I for the description of the model). Kₑᵥ = ([L] × [A]/[LA]) = kₑᵥ/kₙᵥ.
- *Kₑᵥ* is defined as Kₑᵥ = Kₑᵥ × (1 + Kₑᵥ) with Kₑᵥ = kₑᵥ/kₙᵥ and Kₑᵥ = kₑᵥ/kₑᵥ. Kₑᵥ corresponds to the global thermodynamic constant related to the interaction since Kₑᵥ = ([L] × [A]/[LA] + [LA]²). The values given for Kₑᵥ correspond to the mean ± S.D. of n independent experiments.

**Table VII**

| Kinetic parameters | TGF-β1 isofrom | TGF-β2 isofrom | TGF-β3 isofrom |
|--------------------|----------------|----------------|----------------|
| kₑᵥ (M⁻¹ s⁻¹)     | (5.3 ± 0.3) × 10⁴ | (4.6 ± 0.3) × 10⁴ | No binding |
| Kₑᵥ (nM)          | 509 ± 71       | 1126 ± 193    | 50.5 ± 14 (n = 3) |
| Kₑᵥ (no unit)     | (1.35 ± 0.01) × 10⁻¹ | (5.7 ± 0.4) × 10⁻² | |
| Kₑᵥ (nM)          | 60 ± 10 (n = 3) |                    | |

The striking difference in the abilities of the type II ED versus the type II ED/Fe chimera to sequester TGF-β and thus inhibit signaling (Fig. 7, C and D) can be directly correlated with their apparent dissociation rates (compare the wash-off phases in Figs. 2 and 4) and kₑᵥ from Table V with kₑᵥ from Table VII), but not with their thermodynamic dissociation constants (~100 nM) (see Tables V and VII). These results indicate that the apparent dissociation rate, and not the thermodynamic dissociation constant, may be the parameter that best explains the antagonistic potential of a TGF-β-binding protein. In the case of the type II ED/Fe chimera, the decrease in the apparent off-rate is likely due to an effect that appears as a rearrangement of the complex (i.e., the complex with two sites interacting rearranges to a complex with one site interacting; the slow off-rate is reflected in a slow kₑᵥ in Table VII).

With respect to the antagonistic potency of the type III ED and LAP, we propose that, as for the type II ED and the type II ED/Fe chimera, their potency can be explained, at least in part, by their apparent dissociation rate. That is, we are suggesting...
that they neutralize TGF-β relatively effectively (in the 1–100 nM range depending on the binding protein/TGF-β isoform combination) (Fig. 7, A and B) due to the fact that they have relatively slow apparent dissociation rates (compare wash-off phases in Fig. 1–4). In the case of LAP, these slow apparent dissociation rates are reflected in the $k_{d,2}$ dissociation constants (Table IV) and are visually obvious (Fig. 1). In the case of the type III ED, as discussed above, the values of the constants derived from the avidity model are difficult to interpret; however, a visual evaluation of the wash-off phase for the type III ED/TGF-β interaction (Fig. 3) illustrates that it is biphasic, with an obviously slow component. The fact that the type III ED antagonizes as well as it does could be considered surprising due to the fact that the type II and III EDs bind to TGF-β in a non-competitive manner (Fig. 6, D and E), i.e. the type III ED-bound ligand should still be able to bind to cell-surface type II receptors. The antagonistic potency of the type III ED may be explained by our data showing that an interaction between the cytoplasmic domains of the type II and III receptors is critical for enhancement of TGF-β signaling (41).

Together, these results suggest an active role for the type III receptor in signaling in addition to concentrating TGF-β at the cell surface.

The above discussion suggests that the dissociation rate may be used to explain the antagonistic potency. However, in the case where the dissociation rate is held constant, as occurs when comparing the LAP interaction with the three TGF-β isoforms ($k_{d,2}$ varies <2-fold depending on the isoform) (Table IV), it becomes apparent that the association rate ($k_{a}$) also has to be taken into consideration. A comparison of the on-rates for LAP interacting with the three TGF-β isoforms reveals that LAP interacts with TGF-β with a 3–10-fold faster $k_{a}$ compared with TGF-β1 and TGF-β2 (Table IV). The variation in this kinetic constant correlates with the variation in the antagonistic potency of LAP for the three isoforms, i.e. LAP neutralizes TGF-β more effectively than the two other isoforms (Fig. 7A). Hence, although kinetic parameters are explanatory, no single kinetic parameter correlates with antagonistic potency in all cases.

In addition to providing an explanation for the antagonistic potency of soluble TGF-β-binding proteins, the BIAcore data also enable us to construct a model of the cell-surface ligand-occupied TGF-β receptor complex. Based on the stoichiometry of binding that was determined using the BIAcore, we propose that the cell-surface ligand-receptor complex is composed of one type III receptor, two type II receptors, and four type II receptors (Fig. 8). It is likely that this complex recruits four type I receptors based on the recently reported structure of bone morphogenetic protein-2 in complex with two bone morphogenic protein type IA receptor extracellular domains (42). One of the most salient features of this model relative to ones suggested previously (36) is that the type III receptor is proposed to produce the formation of a higher order complex of type I and II receptors. In other words, without the type III receptor, the complex would consist of one ligand and two type I and two type II receptors. In the presence of the type III receptor, the number of ligand and type I and II receptor molecules that are held in close proximity would double. This model suggests that the type III receptor plays an important role not only in concentrating ligand at the cell surface, as has been previously suggested, but also in assembling a potentially more productive type I receptor-type II receptor-ligand complex.

This may explain the finding that the responsiveness of certain cells to exogenous TGF-β1 depends entirely on the presence of the type III receptor (43). Also, the type III receptor has been shown to have an essential role in mediating the effects of TGF-β on mesenchymal transformation in chick embryonic heart development (44). Additionally, we have recently shown that the type III receptor cooperates in the down-regulation of the type I receptor-type II receptor-TGF-β complex (45), again pointing to an important role for the type III receptor in the formation of the type I receptor-type II receptor-ligand complex.

In summary, we have shown that BIAcore data can be used to derive kinetic constants that can explain the antagonistic potency of soluble TGF-β-binding proteins and to determine stoichiometries that allow the construction of a revised model for the cell-surface ligand-occupied TGF-β receptor signaling complex.

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Real-time Monitoring of the Interactions of Transforming Growth Factor-β (TGF-β) Isoforms with Latency-associated Protein and the Ectodomains of the TGF-β Type II and III Receptors Reveals Different Kinetic Models and Stoichiometries of Binding

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