Dimerization of the Extracellular Domain of the Receptor for Epidermal Growth Factor Containing the Membrane-spanning Segment in Response to Treatment with Epidermal Growth Factor*

(Received for publication, April 13, 1999, and in revised form, July 9, 1999)

Kirk G. Tanner† and Jack Kryte‡

From the Department of Chemistry 0506, University of California at San Diego, La Jolla, California 92039-0506

A recombinant fragment of the human receptor for epidermal growth factor containing both its extracellular domain and its membrane-spanning segment, when dissolved with Triton X-100, was observed to dimerize in response to addition of epidermal growth factor (EGF) even at the lowest concentration of this fragment that could be assayed (4 nm). Consequently, the dissociation constant for the dimer of this fragment is at least 10,000-fold smaller than that for dimers of soluble, recombinant forms of the extracellular domain lacking the membrane-spanning segment. The second-order rate constant for dimerization of the fragment containing the extracellular domain and the membrane-spanning segment was estimated to be greater than 0.3 nm⁻¹ min⁻¹, more than 10-fold that of the native enzyme under the same conditions. This result suggests that the cytoplasmic domain of the intact enzyme sterically hinders its dimerization. When EGF is removed from the dimer of the fragment, the rate constant for its dissociation is greater than 0.2 min⁻¹, more than 40-fold that of the native enzyme. This result suggests that interfaces between cytoplasmic domains of intact EGF receptor impart significant stabilization to the dimer of the enzyme.

Experimental Procedures

Preparation of a Detergent Extract from B82 Cells—Cells of the murine B82 line expressing a recombinant form of EGF receptor that is missing its cytoplasmic domain and contains only the extracellular domain and the membrane-spanning segment were graciously provided by Dr. Gordon Gill, Department of Medicine, University of California at San Diego. These cells were grown to confluence, the plates were scraped to release the cells, and the resulting suspension was spun at 3,000 rpm for 30 min in a Sorvall SS-34 rotor at 4 °C. The pelleted cells were lysed (1) by the addition of 4-fold of the pelleted cell volume of 1 mM EDTA, 5 mM ethylene glycol-bisβ-aminoethyl ether-N,N,N',N'-tetraacetic acid, 5 mM 2-mercaptoethanol, 2 mM benzamidinium chloride, 0.1 mM phenylmethylsulfonyl fluoride, 2.5 µg ml⁻¹ aprotinin, 5 µg ml⁻¹ leupeptin, and 20 mM HEPES, pH 7.4. An equal volume of 2% Triton X-100, 20% glycerol, and 30 mM HEPES, pH 7.4, was added to the cell lysates, and the mixture was homogenized with 15–20 strokes in a Dounce homogenizer at 0 °C and then clarified by centrifugation at 100,000 rpm for 30 min at 4 °C in a TLA-100.2 rotor in a Beckman T-100 table top ultracentrifuge. The resulting extracts are referred to as detergent extracts of B82 cells. They were either used immediately or stored for future use at −70 °C. All dilutions of the detergent extract were made in 1% Triton X-100, 30 mM HEPES, pH 7.4.

Assay for Dimerization of Mutant Forms of EGF Receptor in Detergent Extracts of B82 Cells—The states of dimerization of the two truncated forms of EGF receptor were determined by subjecting solutions containing these proteins to quantitative cross-linking with glutaraldehyde (1). Solutions of the purified soluble, recombinant extracellular domain of human EGF receptor (10) in 0.1 M NaCl, 0.5 mM dithiothreitol, 20 mM HEPES, pH 7.4, were graciously provided by Dr. Deborah Cadena, Department of Medicine, University of California at San Diego. These solutions were diluted to the desired concentration of protein with 30 mM HEPES, pH 7.4. Samples (0.3 ml) of the soluble, recombinant extracellular domain or of detergent extracts of B82 cells were mixed at room temperature with 0.15 ml of EGF in 150 mM NaCl, 0.1 mM EDTA, and 20 mM sodium phosphate, pH 7.2 (phosphate-buffered saline) or with phosphate-buffered saline alone to produce the noted final concentrations. The samples then stood at room temperature. Portions were removed at the noted times and cross-linked with glutaraldehyde (final concentration of 80 mM) for 1 min prior to quenching the cross-linking reaction with glycine (final concentration of 0.2 M). The proteins in the samples were unfolded in a solution of recrystallized SDS at 5 g SDS (g protein)⁻¹ for 1 min at 100 °C prior to submitting them to electrophoresis on 6% polyacrylamide gels cast in a solution of 0.1% SDS (11). The cross-linked polypeptides were then electrotransferred (12) to membranes of polyvinylidene difluoride and immunostained (13). For immunostaining of blots of detergent extracts of B82 cells, a mouse monoclonal immunoglobulin (Genosys Biotechnologies, Inc.) raised against the synthetic peptide IQCAHYIDGPHC, corresponding to a region near the juxtamembrane section of the extracellular domain of EGF receptor, was used as the primary immunoglobulin. The intensity of the staining on immunoblots was quantified by scanning them with a reflectance densitometer (1). Assay for the Concentration of Binding Sites for EGF of Extracellular Domain of EGF Receptor Containing the Membrane-spanning Seg-

*This research was supported by Grant GM-47968 and Training Grant DK-07233 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†Present address: Dept. of Biochemistry and Molecular Biology L224, 3181 SW Sam Jackson Park Rd., Portland, OR 97201-3098.
‡To whom correspondence should be addressed: Dept. of Chemistry 0506, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92039-0506. Tel.: 619-534-3281; Facsimile: 619-534-4864; E-mail: jek@chem6.ucsd.edu.
§The abbreviation used is: EGF, epidermal growth factor.
MATERIALS AND METHODS—The assays for the concentration of binding sites for EGF were performed as described by Sherrill & Kyte at a concentration of EGF of 3.3 μM (14). Several different concentrations of protein were assayed as well as a control with no protein. The counts per minute of specifically bound EGF were usually 10-fold greater in the samples with the highest concentration of protein than those for nonspecifically bound EGF in the control samples. When 125I-EGF was used as the radioactive tracer, the supernatant fluids were removed, and both the pellets and the supernatants were submitted to γ-counting. In some samples, unla- beled EGF was added at a higher concentration than that of the 125I- EGF to demonstrate that the bound radioactivity could be chased by unmodified ligand.

RESULTS

Dimerization of the Extracellular Domain Containing the Membrane-spanning Segment—A recombinant extracellular domain of human EGF receptor containing the membrane-spanning segment has been expressed in murine B82 cells (9). This truncated version of EGF receptor contains the amin- terminal portion of the protein through arginine 647. It includes the entire membrane-spanning segment of the protein but does not include more than about six residues of the cyto- plasmic domain. It was dissolved in a solution of Triton X-100 and tested to see if it could undergo dimerization in a manner dependent on the concentration of EGF as does the intact enzyme (1). Samples of a detergent extract of B82 cells were mixed at room tempera- ture with the indicated concentrations of EGF for 20 min prior to subjecting each sample to quantitative cross-linking with glutaraldehyde. The polypeptides in the samples were then separated by electrophoresis on polyacrylamide gels, electrotransferred to a membrane of polyvinylidene difluoride, and immunostained. The recombinant extracellular domain of EGF receptor containing the membrane-spanning segment was observed to dimerize readily in response to EGF (Fig. 1). In lanes 5, 6, 7, and 8 of Fig. 1, the fractions of the protein that had dimerized were 0.09, 0.33, 0.44, and 0.55, respectively. The fraction of the protein that had dimerized was plotted as a function of the concentration of EGF for all of the data from experiments of this type, and the combined data were fit with a rectangular hyperbola. The apparent dissociation constant for EGF, as judged by the concentration of EGF producing half maximum dimerization, was found to be 500 ± 150 nM. In all of the experiments examining the dimerization of the recombi- nant extracellular domain containing the membrane-spanning segment, the maximum extent of dimerization observed at saturation with EGF was about 0.45 to 0.50. This result indi- cates that in the B82 cells, the expressed protein is present in at least two forms, one competent to dimerize, the other incom- petent.

Experiments were performed to monitor the dimerization of the extracellular domain of EGF receptor containing the membrane-spanning segment as a function of time. Samples of detergent extracts of B82 cells were mixed at room tempera- ture with either phosphate-buffered saline or a saturating concentra- tion of EGF in phosphate-buffered saline. At the noted times, samples were assayed for dimerization. The extracellular domain of EGF receptor containing the membrane-spanning segment in undiluted detergent extracts of B82 cells dimerized to the maximum extent within the 1 min resolution of the cross-linking reaction (Fig. 2). The fractions of the protein that had dimerized in the experiment presented in Fig. 2 were 0.47, 0.45, 0.46, and 0.45 at 1 min, 3 min, 5 min, and 10 min, respectively.

Detergent extracts of B82 cells were diluted in an attempt to slow the presumably second-order dimerization of the extracel- lular domain of EGF receptor containing the membrane-span- ning segment. Several dilutions (3-fold, 5-fold, 10-fold, and 20-fold) of the detergent extract of B82 cells were attempted. In each case, the dimerization of the extracellular domain of EGF receptor containing the membrane-spanning segment still pro- ceeded too rapidly. At a dilution of 20-fold, the fractions of the protein that had dimerized were 0.44, 0.51, 0.50, and 0.49 at 1 min, 3 min, 5 min, and 10 min, respectively (Fig. 3).

The molar concentration of extracellular domain of EGF receptor containing the membrane-spanning segment in the undiluted detergent extract of B82 cells used in this last experiment was estimated to be 150 nM on the basis of an assay for the binding of EGF (14). If it is assumed that the dimerization is greater than 50% complete in less than 1 min (Fig. 3) at a concentration of protein competent to dimerize of 2.5 nM, the second-order rate constant for the dimerization of the protein must be greater than 0.3 nM⁻¹ min⁻¹. Under identical conditions of pH, temperature, and concentrations of glycerol and Triton X-100, the rate constant for the dimerization of intact native EGF receptor is 0.03 nM⁻¹ min⁻¹ (1, 14). Therefore, the extracellular domain of EGF receptor containing the membrane-spanning segment dimerizes at a rate more than 10-fold faster than that for the dimerization of native, intact EGF receptor.

In an attempt to slow the dimerization of the extracellular domain of EGF receptor containing the membrane-spanning segment even further, EGF was added to samples of a deter- gent extract of B82 cells at 0 °C. Even at the lower tempera- ture, undiluted samples of the extract still dimerized com- pletely within 1 min of the cross-linking reaction. Epidermal growth factor was then added to a sample of a detergent extract
of B82 cells that had been diluted 10-fold and brought to 0 °C. The rate of dimerization of the extracellular domain of EGF receptor containing the membrane-spanning segment in these samples standing on ice was decreased sufficiently to observe the dimerization proceed over the course of 20 min (Fig. 4).

Rate of Dissociation of the Dimerized Extracellular Domain—In a previous study (15), it was found that when intact, native EGF receptor was activated with saturating EGF to produce a fully dimerized and enzymatically active state and then passed over a column containing an immunoadsorbent made from anti-EGF immunoglobulins that could remove both the EGF in solution and any bound EGF, the unoccupied EGF receptor nevertheless remained dimerized and enzymatically active over extended periods of time. The rate of dissociation the dimerized recombinant extracellular domain of EGF receptor containing the membrane-spanning segment was assessed in undiluted detergent extracts of B82 cells that had been exposed to EGF for 5 min at room temperature prior to removing the EGF by passing the sample over the immunoadsorbent for EGF. Samples of a pool of the fractions of the eluate from the immunoadsorbent containing the protein were subjected to quantitative cross-linking with glutaraldehyde at various times over a period of 80 min (Fig. 5). The dimerized extracellular domain of EGF receptor containing the membrane-spanning segment had reverted completely to its monomeric form within 5 min following the removal of the EGF (lane 1). The readdition of EGF after its removal, however, was able to return the protein to its dimeric form (Lanes 5–8). This control shows that the immunoadsorbent had not affected the ability of the protein to dimerize but had merely removed the EGF. From these results, it can be estimated that the rate constant for the monomerization of the dimeric extracellular domain containing
A sample was applied to the immunoadsorbent for EGF and allowed to span the extracellular domain of EGF receptor containing the membrane-spanning segment. After standing for 5 min at room temperature, the sample was applied to the immunoadsorbent for EGF and allowed to remain there for 2 min before the protein was eluted with 10% glycrol, 0.05% Triton X-100, 1 mM EDTA, 6 mM 2-mercaptoethanol, 10 μg ml⁻¹ leupeptin, and 30 mM HEPES, pH 7.4. Fractions of the eluate containing the protein that had been depleted of EGF were pooled and split into two equal samples. To one of these samples (lanes 1–4) phosphate-buffered saline was added; to the other (lanes 5–8), EGF was added back to 830 nM. The samples then stood at room temperature, and portions were removed at the noted times and cross-linked with 80 μl glutaraldehyde for 1 min before quenching the cross-linking reaction with 0.2 M glycine. The polypeptides were then dissolved by adding SDS, subjected to electrophoresis, electrotransferred, and immunostained. Lanes 1–4 are of the sample to which phosphate-buffered saline had been added after the immunoadsorption and from which portions were cross-linked with glutaraldehyde at 10, 20, 40, and 80 min, respectively, after the initial addition of EGF before the immunoadsorption. Lanes 5–8 are of the sample to which EGF had been added back after the immunoadsorption and from which portions were cross-linked at 13, 23, 43, and 83 min after the initial addition of EGF.

The membrane-spanning segment, following removal of the EGF, is greater than 0.2 min⁻¹. This value is more than 50-fold greater than that of the rate constant (0.26 h⁻¹) for the monomerization of dimeric native EGF receptor following the removal of EGF by the same immunoadsorbent under the same conditions (15).

**Dimerization of Soluble, Recombinant Extracellular Domain**—In previous reports (5), it has been demonstrated that soluble, recombinant forms of the extracellular domains of EGF receptor must be at high concentrations (40 μM or greater) to display dimerization. It was possible, however, that the conditions we were using to examine the dimerization of the extracellular domain containing the membrane-spanning segment, which occurred readily even at very low concentrations (2.5 nM), somehow dramatically enhanced the dimerization of the extracellular domain. Therefore, experiments were performed to assess the ability of a soluble, recombinant extracellular domain of EGF receptor to dimerize in response to treatment with EGF under the same conditions of pH, detergent concentration, and temperature as the experiments just described. The soluble, recombinant extracellular domain used in these experiments was the one expressed in Chinese hamster ovary cells by Cadena & Gill (10), which included the amino-terminal domain of human EGF receptor through threonine 624. This protein was a gift of Dr. Deborah Cadena, who had purified it by chromatography on Sepharose to which wheat germ agglutinin had been attached. Each preparation had been routinely assayed by Dr. Cadena for the binding of EGF which displayed an apparent dissociation constant of 500 ± 200 nM (10). The concentrations of the soluble, recombinant extracellular domain of EGF receptor used in these assessments of its ability to dimerize spanned a range of 1,000-fold (5 nM to 5 μM). In some experiments the concentration of the soluble, recombinant extracellular domain of EGF receptor was high enough (5 μM) to visualize it on polyacrylamide gels by staining them with Coomassie Brilliant Blue. Epidermal growth factor was incubated with the soluble, recombinant extracellular domain of EGF receptor at final concentrations as high as 30 μM, which is 60-fold higher than its apparent dissociation constant. Both quantitative cross-linking with glutaraldehyde (1) and cross-linking with disuccinimidyl suberate (7) were performed. Most of these experiments were carried out in the absence of detergent, but in two experiments, one at 5 μM in soluble, recombinant extracellular domain, Triton X-100 and glycerol were added to 1 and 10%, respectively, to mimic more closely the conditions under which the detergent extracts of B82 cells expressing the soluble, recombinant extracellular domain of EGF receptor containing the membrane-spanning segment were examined. Experiments were also performed in which the cross-linking reaction with glutaraldehyde was extended from 1 to 10 min. No dimerization of the soluble, recombinant extracellular domain of EGF receptor induced by the addition of EGF was detected under any of these conditions. The concentration of EGF that was incubated with the soluble, recombinant extracellular domain of EGF receptor in these studies was always well above the reported dissociation constant of 500 nM.

Using the same preparation of soluble, recombinant extracellular domain of EGF receptor, Cadena & Gill (10) also could not detect any EGF-induced dimerization, as determined by cross-linking with glutaraldehyde, at 1.5 μM protein and 1.7 μM EGF. Neither our results nor those of Cadena & Gill (10) are surprising, however, because concentrations of 45 μM or greater of a similar recombinant extracellular domain of EGF receptor were needed to observe dimerization in the experiments of Lemmon et al. (5).

**DISCUSSION**

To define more precisely the regions of EGF receptor that may form contacts that are important in its dimerization, a recombinant form of the extracellular domain of EGF receptor containing the membrane-spanning segment of the protein (9) dissolved in a solution of Triton X-100 was examined to see if it would dimerize in response to addition of EGF. Not only did this fragment of the protein dimerize in response to treatment with EGF, but it did so at a rate more than 10-fold greater than that for the dimerization of intact native EGF receptor when the two proteins are assayed at the same concentration of EGF. This result suggests that regions of the cytoplasmic domain of native, intact EGF receptor interfere with its dimerization.

It has been difficult to detect dimerization of soluble, recombinant forms of the extracellular domain of EGF receptor lacking the membrane-spanning domain. Several other investigators have had experiences similar to ours in being unable to detect dimerization of soluble, recombinant extracellular domains of human EGF receptor (20, 21). In other studies, however, it has been concluded that EGF is able to promote dimerization of the soluble, recombinant extracellular domain of EGF receptor when that domain is present at high concentrations. Hurwitz et al. (22) were able to detect small amounts of dimerization of a soluble, recombinant extracellular domain of EGF receptor produced in baculovirus by cross-linking with disuccinimidyl suberate. Under these conditions an almost equivalent amount of trimer and detectable amounts of tetramer were also observed (7, 22). The long cross-linking reaction with disuccinimidyl suberate at the high concentrations of EGF (0.1 mg ml⁻¹) may have resulted in the cross-linking of the EGF itself, forming dimers, trimers, and tetramers, and these multivalent forms of the hormone may have led to the higher oligomers of the extracellular domain that were observed. In a separate study, Brown et al. (23) presented data interpreted as demonstrating that the binding of EGF could induce dimerization of a soluble, recombinant extracellular...
domain of EGF receptor, but the concentration of protein used (170 μM) was so high that significant amounts of dimer were detected even in the absence of EGF, whereas incubation with high concentrations of EGF (340 μM) were required to produce increases in the yield of dimer and even then the monomer still predominated. The most striking experimental observation in all of these studies was that the concentrations of EGF required to observe significant amounts of the dimeric recombinant extracellular domain were anywhere from 100-fold to more than a 1,000-fold higher than the reported dissociation constants (22, 23). One reason that such high amounts of EGF had to be used in some of these experiments was that the soluble, recombinant extracellular domain had to be at high concentrations to observe the dimerization. Nevertheless, one is left with the impression that the oligomerization that is occurring during the long times involved in the cross-linking of EGF receptor performed in many of these experiments is only indirectly related to the binding of EGF. The recent report by Lemmon et al. (5), however, is significantly more convincing. They report that EGF induces the quantitative formation of dimers of a soluble, recombinant extracellular domain of EGF receptor, as assayed by small angle x-ray scattering, but only at high concentrations of the protein. This has been the only report that has demonstrated a quantitative dimerization of a soluble, recombinant extracellular domain of EGF receptor.

Dimerization of the extracellular domain containing the membrane-spanning segment proceeded to completion at concentrations of competent protein as low as 2.5 nM (Fig. 3). Under identical conditions of pH, ionic strength, temperature, and concentrations of glycerol and Triton X-100, intact, native EGF receptor at concentrations as low as 2 nM also dimerizes completely upon addition of EGF (1, 14). In contrast, to observe quantitative yields of dimerization of a soluble, recombinant extracellular domain lacking the membrane-spanning segment, Lemmon et al. (5) used concentrations of the domain of 45 μM or greater. Consistent with their results, we and Cadena & Gill (10) were unable to observe any dimerization of a similar soluble, recombinant extracellular domain at concentrations as high as 5 μM.

The fact that the apparent dissociation constant for EGF of the extracellular domain containing the membrane-spanning segment, as judged by its effect on the yield of dimer (Fig. 1), and the apparent dissociation constant for the soluble, recombinant extracellular domain lacking the membrane-spanning segment, as judged by cross-linking [125I]iodinated EGF to its binding site, were both 500 nM suggests that comparisons of these two proteins should be meaningful. It is also reassuring that these values for the apparent dissociation constants compare favorably with values of 350 nM (21), 250 nM (7), and 150 nM (22) for other preparations of soluble, recombinant EGF receptor. Nevertheless, it must be kept in mind that the conditions under which the dimerization of the soluble, recombinant extracellular domain and the dimerization of the extracellular domain containing the membrane-spanning segment were assessed in the present experiments were not identical because these two proteins were prepared by different protocols. The soluble, recombinant extracellular domain was provided to us as a protein that had been purified from the culture medium surrounding the Chinese hamster ovary cells that had excreted it, and the extracellular domain containing the membrane-spanning segment was expressed in murine B82 cells and tested for dimerization in the context of a crude lysate of these cells. We did examine these two proteins under identical conditions of temperature, pH, and detergent concentration. In fact, even at a 20-fold dilution, the condition most important for its comparison with the soluble, recombinant extracellular do-

main, the extracellular domain containing the membrane-spanning segment showed no decrease in the amount of dimerization (Fig. 3). Under these conditions, the remaining cytoplasm of the lysed cells had been diluted more than 200-fold over its concentration in the cell, and the solution should be even more similar to that in which the purified soluble, recombinant extracellular domain was dissolved. For all of these reasons, we believe that valid comparisons can be made between the behavior of these two proteins.

Therefore, dimers of both intact, native EGF receptor and of the extracellular domain containing the membrane-spanning segment have dissociation constants at least 10,000-fold smaller than that of the soluble, recombinant extracellular domain. It follows that addition of the segment of the 23 amino acids GMVGALLLLLVVALGIGLFMRRR, which contains the sequence spanning the membrane with an additional three to six amino acids that extend into the cytoplasm, converts a protein that dimerizes reluctantly into a protein that dimerizes avidly in response to the addition of EGF.

This observation suggests that rather than playing a passive role in the dimerization of EGF receptor produced by EGF, the two membrane-spanning domains from the two monomers of EGF receptor that dimerize upon the binding of EGF provide molecular surfaces that associate to yield a significant portion of the standard free energy of dimerization. This possibility has always been an implication of the fact that the mutation of valine 664 to a glutamate (17) in the membrane-spanning segment of the rat Neu protein turns an inactive, monomeric protein tyrosine kinase into a constitutively active (18), constitutively dimeric (19) protein tyrosine kinase.

It has been argued that the high concentrations of soluble, recombinant extracellular domain that are necessary to observe dimerization result from the fact that the protein is in free solution rather than in the membrane (5). While it is true that intact EGF receptor in a cell membrane is restricted to diffusion in two, rather than three, dimensions, native intact EGF receptor or its extracellular domain containing the membrane-spanning segment when they are dissolved in solutions of Triton X-100 have the same degrees of diffusional freedom as a soluble, recombinant extracellular domain of EGF receptor, yet high concentrations of these proteins are not required to observe the dimerization produced by EGF. Because a 1% solution of Triton X-100 is 0.1 μl in micelles (24, 25), a concentration over 1,000-fold greater than the concentration of native EGF receptor in even undiluted detergent extracts of A431 cells, it can be concluded that the small minority of the micelles that are occupied by molecules of native EGF receptor or the extracytoplasmic domain containing the membrane-spanning segment will contain only one molecule of the respective monomeric protein. In fact, if a significant fraction of the molecules of unactivated monomeric intact EGF receptor or monomeric unactivated extracellular domain containing the membrane-spanning segment shared the same micelle with any protein, let alone one of its twins, then cross-linking of it with these other molecules of protein would be unavoidable because of their close proximity, and clear bands of immunostained monomeric protein would not be observed on the respective Western blots, but they are (1). If each molecule of monomeric native EGF receptor in these solutions is alone in its micelle, there is no fundamental difference between its diffusion through the solution and the diffusion of the soluble, recombinant extracellular domain through the solution, and direct comparisons between the two in terms of dissociation constants are valid.

Nor is it proper to consider the micelles of Triton X-100 as a separate phase (5) when evaluating the dissociation constants
of the intact native EGF receptor or its extracellular domain containing the membrane-spanning segment. The proteins are equal to or greater in mass (molar masses of 71,300 g mol\(^{-1}\) and 132,000 g mol\(^{-1}\)) than a micelle (24, 25) of Triton X-100 alone (molar mass of 90,000 g mol\(^{-1}\)). Furthermore, the size of a micelle of Triton X-100 does not dramatically increase as phospholipid dissolves in it (24, 26); in the relevant range of mole fraction, as molecules of phospholipid are incorporated into a micelle, the number of molecules of Triton X-100 present increases at most by 50% (24). Therefore, the mixed micelles of Triton X-100 and endogenous phospholipid surrounding the dissolved transmembrane segments of the proteins in the detergent extracts used in these experiments are simply accretions upon the proteins themselves (27). Consequently, the molar concentrations relevant to quantification of the equilibria are those of the complexes between the proteins and the detergent expressed in moles of complex for each liter of aqueous solution, not in moles for each liter of Triton X-100.

Finally, it is unlikely that the simple fusion of the two micelles, one from each monomer of the native enzyme or of the extracellular domain containing the membrane-spanning segment, contributes any favorable free energy of association to the formation of a dimer. This conclusion follows from the fact that above the critical micelle concentration, increasing the concentration of Triton X-100 has little effect on the mass of either micelles of pure Triton X-100 or mixed micelles of phospholipid and Triton X-100 (24, 25). This observation indicates that a micelle represents the most stable oligomer of the detergent at the given pH, ionic strength, and temperature (16), and doubling its size by fusion should, if anything, be unfavorable. When both free EGF and EGF bound to the extracellular domain of EGF receptor containing the membrane-spanning segment were removed from the solution by passage over an immunoadsorbent for EGF (15), the dimeric protein reverted to monomer within 5 min of the removal of the EGF (Fig. 5). This behavior is distinct from the behavior of dimeric, enzymatically immunoadsorbent for EGF (15), the dimeric protein reverted to monomer. This suggests that the conformational change relieving the steric inhibition is rapidly reversible upon dissociation of EGF. When the cytoplasmic domain of EGF receptor is present, however, additional surfaces from these domains in the dimer of EGF receptor must interact favorably to provide a significant portion of the stabilization of the homodimer because dimerization of the intact native EGF receptor persists for hours following the removal of EGF by passage over the immunoadsorbent.

Acknowledgments—We thank Dr. Gordon Gill, Department of Medicine, University of California at San Diego for providing us with the B-82 cells used in these experiments and for his consistent interest and encouragement. Samples of the purified soluble, recombinant extracellular domain of human EGF receptor were kindly provided by Dr. Deborah Cadena, Department of Medicine, University of California at San Diego.

REFERENCES

1. Canals, F. (1992) Biochemistry 31, 4493–4501
2. Siegel, P. M., and Muller, W. J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 8878–8883
3. Philo, J., Aoki, K. H., Arakawa, T., Narhi, L. O., and Wen, J. (1996) Biochemistry 35, 1681–1691
4. Cohen, B. D., Green, J. M., Fry, L., and Fell, H. P. (1996) J. Biol. Chem. 271, 4813–4818
5. Lemmon, M. A., Bu, Z., Ladbury, J. E., Zhou, M., Pinchasi, D., Lax, I., Engelman, D. M., and Schlessinger, J. (1997) EMBO J. 16, 281–294
6. Ullrich, A., Coussens, L., Hayflick, J. S., Dull, T. J., Gray, A., Tam, A. W., Lee, J., Yarden, Y., Libermann, T. A., Schlessinger, J., Downward, J., Mayes, E. L. V., Whittle, N., Waterfield, M. D., and Stinchcomb, P. H. (1984) Nature 309, 418–425
7. Lax, I., Mirza, A. R., Ravera, C., Hurwitz, D. R., Rubinstein, M., Ullrich, A., Stroud, R. M., and Schlessinger, J. (1991) J. Biol. Chem. 266, 13826–13833
8. Wedeguertner, P. B., and Gill, G. N. (1989) J. Biol. Chem. 264, 11346–11353
9. Chen, W. S., Lazar, C. S., Lund, K. A., Walsh, J. B., Chang, C., Walton, G. M., Channing, J., Der, H., Wiley, S., Gill, G. N., and Rosenfeld, M. G. (1989) Cell 59, 33–43
10. Sisk, B. A., and Gill, G. N. (1993) Protein Expression Purif. 4, 177–186
11. Laemmli, U. K. (1970) Nature 227, 680–685
12. Towbin, H., Staehelin, L., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354
13. Blake, M. S., Johnston, K. H., Russell-Jones, G. J., and Gotosch, E. C. (1984) Anal. Biochem. 136, 175–179
14. Sherrill, J. M., and Kyte, J. (1996) Biochemistry 35, 5705–5718
15. Tanner, K. (1997) Biochemistry 36, 14889–14896
16. Tanford, C. (1980) The Hydrophobic Effect: Formation of Micelles and Biological Membranes, 2nd Ed., pp. 42–59, John Wiley & Sons, NY
17. Bargmann, C. I., Hung, M., and Weinberg, R. A. (1996) Cell 85, 5495–5498
18. Weinman, D. B., Liu, J., Cohen, J. A., Williams, W. V., and Greene, M. I. (1989) Nature 339, 230–231
19. Gunther, N., Betzel, C., and Weber, W. (1990) J. Biol. Chem. 265, 22082–22085
20. Greenfield, C., Hils, I., Waterfield, M. D., Pederwich, M. L., Wolmer, A., Blundell, T. L., and McDonald, N. (1989) EMBO J. 8, 4115–4123
21. Hurwitz, D. R., Emanuel, S. L., Nathan, M. H., Sarver, N., Ullrich, A., Felder, S. L., and Schlessinger, J. (1991) J. Biol. Chem. 266, 22835–22843
22. Brown, P. M., Dhaune, M. T., Grothe, S., Bergema, D., Cavar, M., Ray, C., and O'Connor-McCourt, M. D. (1994) Eur. J. Biochem. 225, 223–233
23. Yedgar, S., Barenholz, Y., and Cooper, V. G. (1974) Biochim. Biophys. Acta 323, 98–111
24. Kushner, L. M., and Hubbard, W. D. (1954) J. Phys. Chem. 58, 1163–1167
25. Robson, R. J., and Dennis, E. A. (1979) Biochim. Biophys. Acta 573, 489–500
26. Kyte, J. (1985) Structure in Protein Chemistry, pp. 521–523, Garland, NY