CONTRASTING INTERLEUKIN 2 BINDING PROPERTIES OF THE $\alpha$ (p55) AND $\beta$ (p70) PROTEIN SUBUNITS OF THE HUMAN HIGH-AFFINITY INTERLEUKIN 2 RECEPTOR

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Receptors for the polypeptide growth factor IL-2 are expressed on the cell surface of activated T and B cells and can be resolved into high-affinity ($K_d \sim \text{5–50 pM}$) and low-affinity ($K_d \sim \text{1–30 nM}$) forms (1-3). Recent experimental evidence (1, 2) strongly suggests that the growth-promoting effect of IL-2 is mediated by the high-affinity IL-2-R, whereas no biological function has yet been attributed to the 10-30-fold more abundant low-affinity IL-2-R. IL-2-R of the high-affinity class can be distinguished from those of low affinity both by their ability to rapidly internalize IL-2 at $37^\circ\text{C}$ (4-6) and by a ~100-fold slower rate of IL-2 dissociation (7).

The structural difference in the high- and low-affinity classes of IL-2-R is not yet resolved, but the $M_r$ 55,000 (p55) IL-2-binding glycoprotein (also referred to as Tac) has been shown to be a component of both forms of the receptor (2, 8). Recently, a second IL-2-binding protein ($M_r$ of 70,000–75,000, p70), distinct from the p55 antigen, has been characterized in a number of laboratories (9–14). The p70 protein is not recognized by antibodies reactive with different epitopes of the p55 protein (11–13) and appears to bind IL-2 with intermediate affinity ($K_d \sim \text{0.5–1 nM}$) (11–13) at an epitope distinct from that recognized by the p55 antigen (12). The p70 protein, unlike p55, is also capable of mediating rapid internalization of IL-2 (14). $^{125}$I-IL-2 crosslinking experiments (9–13) and reconstitution studies (11–13, 15) suggest that the high-affinity IL-2-R corresponds to a membrane complex composed of at least the p55 and p70 proteins. Recently, a number of T cell lines have been identified that independently express either the p55 or p70 chain in the absence of the other subunit (10–15). We have used these cells to dissect the kinetics of IL-2 binding to the individual p55 and p70 chains and to compare and contrast these results with ligand binding to the high-affinity IL-2-R complex believed to be composed of both chains. Based on their order of identification, Sharon et al. (9) originally proposed that the p55 and the p70 proteins be referred to as the $\alpha$ and $\beta$ chains, respectively, of the putative high-affinity IL-2-R complex. However, based on the size of the proteins, the opposite assignment of $\alpha$ and $\beta$ has also been suggested (11). In this manuscript, we have used the original nomenclature, but to avoid confusion we also identify each of the chains in parentheses as p55 or p70.
Materials and Methods

**Cells.** The HUT 102B2, MT-1, MLA-144, and YT cell lines were used in this study. HTLV-1-infected HUT 102B2 cells express both high- and low-affinity IL-2-R. At low ligand concentrations (i.e., 20 pM), IL-2 predominantly binds to high-affinity IL-2-R, while at high ligand concentrations (i.e., 1 nM), IL-2 is primarily bound to the more numerous low-affinity IL-2-R. MT-1 is a second HTLV-1-transformed T cell line; however, unlike HUT 102B2 cells, they express only low-affinity IL-2-R (5) and no detectable \( \alpha \) (p70) chains (10, 13-15). In contrast, the MLA-144 gibbon T cell line expresses \( \beta \) (p70) chains in the absence of \( \alpha \) (p55) chains (10, 13) and binds IL-2 with an intermediate affinity (13). YT is a T cell line of immature phenotype that expresses a relatively large number of \( \beta \) (p70) chains in the presence of a very small number of \( \alpha \) (p55) chains (12). The binding of IL-2 to the few high-affinity IL-2-R on these cells can be eliminated by the addition of 500 nM anti-Tac antibody (12 and data not shown).

**Reagents.** Purified human rIL-2 was generously provided by Cetus Corp. (Emeryville, CA). Iodinated rIL-2 (\(^{125}\)I-IL-2, 43.2 \( \mu \)Ci/\( \mu \)g) was obtained from New England Nuclear, Boston, MA.

**Determination of IL-2 Association and Dissociation Kinetics.** The binding of \(^{125}\)I-IL-2 was measured as previously described (1-3, 7). To determine the kinetics of IL-2 association, aliquots of cells were incubated with \(^{125}\)I-IL-2 (20 pM or 1 nM) in a total volume of 200 \( \mu \)l for varying periods of time at 4\(^{\circ}\) or 37\(^{\circ}\)C. Nonspecific binding was determined by the addition of a 500-fold molar excess of unlabeled IL-2. To measure the kinetics of IL-2 dissociation, the cells were first incubated with \(^{125}\)I-IL-2 for 1-3 h at 4\(^{\circ}\)C. A 500-fold excess of unlabeled IL-2 was then added and the amount of cell-bound \(^{125}\)I-IL-2 was determined after varying times of incubation.

Results and Discussion

A summary of IL-2-R number and binding affinities for the HUT 102B2, YT, MLA-144, and MT-1 cell lines is presented in Table I. Using these cell lines, the kinetics of IL-2 association and dissociation to the \( \alpha \) (p55) and \( \beta \) (p70) chains and to the high- and low-affinity IL-2-R were studied. IL-2 binding kinetics to the \( \beta \) (p70) chain present on YT and MLA-144 cells are shown in Fig. 1, A and B. IL-2 association with the \( \beta \) (p70) chain occurred slowly at 4\(^{\circ}\)C, with a \( t_{1/2} \) value of 42 min for YT cells and 47 min for MLA-144 cells (Fig. 1A). For both cell lines, steady-state binding was only achieved after 3-4 h of incubation with the ligand. The rate of IL-2 dissociation from the \( \beta \) (p70) protein (Fig. 1B) was also found to occur slowly, with a \( t_{1/2} \) of 175-220 min.

We next measured IL-2 binding kinetics to the \( \alpha \) (p55) chain expressed on MT-1 cells. In sharp contrast to the slow rate of IL-2 association to the \( \beta \) (p70) chain, the \( \alpha \) (p55) chain rapidly bound IL-2 with a \( t_{1/2} \) of ~4 s (Fig. 1C). Maximum
FIGURE 1. Kinetics of IL-2 association to, and dissociation from the α (p55) and β (p70) chains and high-affinity IL-2-R. The time course of IL-2 association (A, C, E) and dissociation (B, D, F) was measured by incubating the indicated cells in the presence of either 1 nM (A–D) or 20 pM (E, F) 125I-IL-2 at 4°C. Aliquots of cells were removed at the indicated time intervals and the amount of cell bound radioactivity was measured as described in Materials and Methods. In this set of experiments, dissociation of 125I-IL-2 was initiated by the addition of a 300-fold molar excess of unlabeled IL-2. In experiments not shown here, the rate of 125I-IL-2 dissociation was found to be essentially identical when cells were washed and resuspended in IL-2-free medium. The $t_{1/2}$ for IL-2 association and dissociation was calculated from log-linearized plots of the respective time courses. The number of cells used per time point was: $2 \times 10^6$ for MLA-144, $10^6$ for HUT 102B2, and YT (A), and $5 \times 10^5$ for MT-1 and YT (B).

binding occurred within $\sim 20$ s. Dissociation of IL-2 from the α (p55) chain also proceeded at a very rapid rate, with a $t_{1/2}$ of 6 s (Fig. 1D). Fig. 1, C and D, demonstrates that the IL-2 binding kinetics to the low-affinity IL-2-R present on HUT 102B2 are essentially identical to those of the α (p55) chain present on
MT-1 cells. Binding of IL-2 to low-affinity IL-2-R was measured by incubating HUT 102B2 cells in the presence of a high concentration (1 nM) of $^{125}$I-IL-2. Under these conditions, most (~85%) of the cell-associated IL-2 was bound to low-affinity IL-2-R. The subsequent addition of an excess of unlabeled IL-2 resulted in a biphasic pattern of ligand dissociation. The majority of the IL-2 dissociated rapidly with a $t_{1/2}$ value of 9 s, which corresponds to that of the $\alpha$ (p55) chain (Fig. 1D). The remaining IL-2 dissociated at a much slower rate and presumably represented IL-2 bound to high-affinity IL-2-R. Similar results have been reported for IL-2 dissociation from murine high- and low-affinity IL-2-R (7).

Finally, the kinetics of IL-2 binding to the high-affinity IL-2-R was compared with the $\alpha$ (p55) and $\beta$ (p70) chains by incubating HUT 102B2 cells in the presence of low (20 pM) concentrations of $^{125}$I-IL-2. Under these conditions, approximately half of the high-affinity IL-2-R are occupied at equilibrium with virtually no binding to low-affinity receptors. Fig. 1E demonstrates that IL-2 bound rapidly to high-affinity IL-2-R, reaching equilibrium within 2–5 min at 4°C. The $t_{1/2}$ value of 42 s resembled the rapid kinetics of IL-2 binding characteristic of the $\alpha$ (p55) chain and was in marked contrast to the slow rate of binding to the $\beta$ (p70) chain. In contrast to the rapidity of IL-2 association, the rate of IL-2 dissociation from the high-affinity receptor was quite slow with a $t_{1/2}$ of 275 min (Fig. 1F). This slow dissociation was very similar to that determined for the $\beta$ (p70) chain.

The IL-2 association and dissociation kinetics for high-, low-, and intermediate-affinity IL-2-R were next measured at a physiological temperature (37°C). Table II shows that the rate of IL-2 binding to high-affinity IL-2-R and to the $\beta$ (p70) chain each increased two- to sixfold when the temperature was increased from 4°C to 37°C. Notwithstanding, the rate of IL-2 association to the $\beta$ (p70) chain at 37°C remained 80–300-fold slower than the rate of binding to the $\alpha$ (p55) chain. An accurate measurement of the IL-2 dissociation rate at 37°C is difficult to obtain due to concomitant ligand endocytosis mediated both by the $\beta$ (p70) chain (14) and the high-affinity IL-2-R (4–6). However, even after correction for ligand...
internalization, the rate of IL-2 dissociation at 37°C compared with 4°C was five- to sevenfold faster for both the β (p70) chain and high-affinity IL-2-R. In contrast, the rate of IL-2 association to, and dissociation from the α (p55) chain was not significantly different at the two temperatures.

These results reveal several important characteristics of high-, intermediate-, and low-affinity human IL-2-R. The IL-2 binding characteristics of the α (p55) chain are indistinguishable from those of the low-affinity IL-2-R, and strongly suggest that low-affinity receptors correspond to the display of α (p55) chains alone. The extremely fast rate of association for the α (p55) chain suggests that IL-2 binding is probably limited by the rate of IL-2 diffusion. In contrast to the α (p55) subunit, IL-2 binds to the intermediate-affinity β (p70) subunit at a much slower rate and may not be diffusion limited. Rather, these results suggest that a conformational change in the structure of the β (p70) chain may be required for, or involved in, ligand binding. Interestingly, the binding characteristics of the high-affinity IL-2-R combine the most biologically favorable attributes of both chains, namely the rapid IL-2 association rate of the α (p55) chain and the slow IL-2 dissociation rate of the β (p70) chain. These findings thus provide additional support for the proposed hypothesis that the high-affinity IL-2-R corresponds to an α/β heterodimer receptor complex. While the precise nature of the interaction between the α (p55) and β (p70) chains remains to be determined, the very rapid dissociation rate of IL-2 from the α (p55) subunit argues against the possibility that this protein serves only to focus IL-2 onto the β (p70) subunit. Rather, it seems more likely that the association of the α and β subunits results in a conformational change in the structure of either or both of the chains, thereby resulting in the display of a receptor that is uniquely able to bind, retain and endocytose IL-2 before it dissociates from the cell surface. These findings underscore the unique role played by each of these proteins in the formation of functional high-affinity IL-2-R. Further study of these proteins, as well as the possible existence of other, yet unidentified receptor subunits, should provide a clearer understanding of the structure and mechanism of signal transduction by the high-affinity IL-2-R.

Summary

In this report, we have investigated the kinetics of IL-2 binding to the α (p55) and β (p70) IL-2 binding proteins and compared these properties with ligand binding to the high-affinity IL-2-R. The association and dissociation of IL-2 to the α (p55) chain occurred with very rapid kinetics ($t_\text{on} = 4-10$ s). In contrast, IL-2 association to, and dissociation from the β (p70) chain occurred at a greatly reduced rate ($t_\text{on} = 40-50$ min and $200-400$ min, respectively). Measurements of IL-2 binding to the high-affinity receptor revealed an interesting composite of these binding properties with a rapid association rate ($t_\text{on} = 30-45$ s) resembling the α (p55) chain and a slow dissociation rate ($t_\text{off} = 270-300$ min) similar to the β (p70) chain. These findings provide additional support for the model of the high-affinity IL-2-R as a heterodimeric membrane complex composed of both the α (p55) and β (p70) subunits and suggest that high-affinity IL-2 binding may involve a conformational change in structure of either or possibly both of the receptor chains. These results highlight the important and perhaps different role played by each subunit in the formation of functional high-affinity IL-2-R.
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