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EVIDENCE FOR p55-p75 HETERO DIMERS IN THE
ABSENCE OF INTERLEUKIN-2 FROM
SCATCHARD PLOT ANALYSIS*

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Interleukin-2 (IL-2)
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Scatchard Plot
Affinity Conversion Model
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Receptor Aggregation

Abbreviations

Interleukin-2 (IL-2)
MLC (mixed lymphocyte culture)
p55 (light chain of the IL-2 receptor)
p75 (heavy chain of the IL-2 receptor)
PBL (peripheral blood lymphocytes)
PHA (phytohemagglutinin)
ABSTRACT

The high affinity receptor for interleukin 2 (IL-2) is composed of at least two chains, a p55 chain that binds IL-2 with low affinity and a p75 chain that binds with intermediate affinity. Two molecular mechanisms have been proposed for the formation of the high affinity receptor-ligand complex: The affinity conversion model proposes that the high affinity receptor is formed via stepwise binding in which IL-2 first binds to the p55 chain and the resulting complex then associates with the p75 chain to form a high affinity ternary complex. In the preformed heterodimer model the p55 and p75 chains form a non-covalently linked high affinity heterodimer in the absence of IL-2. We show that these two models can be distinguished on the basis of equilibrium binding experiments using cell lines expressing different numbers of p55 chains. To make this distinction we develop a general model for the interaction of IL-2 with its various receptors. We then analyze the case in which heterodimers exist in the absence of IL-2, and the case in which no preformed heterodimers exist. For both cases we predict the shape of equilibrium Scatchard plots. We then show that published IL-2 binding studies are consistent with a model in which a large concentration of preformed heterodimers is present on the cell surface and inconsistent with a model in which preformed heterodimers are absent from the cell surface. The models that we develop should have general applicability to the entire class of receptor systems in which low and intermediate affinity chains interact to constitute a high affinity receptor.
INTRODUCTION

Two polypeptide chains that bind interleukin 2 (IL-2) have been detected on the surface of activated T cells, a low affinity p55 chain (Tac antigen) and an intermediate affinity p75 chain. High affinity binding has been observed on cells that express both p55 and p75 chains (reviewed in (1,2)). When such cells are exposed to an anti-Tac antibody, which does not affect the binding of IL-2 to the p55 chain, high affinity binding is no longer detected (3,4). Inhibition of high affinity binding has also been obtained with anti-p75 antibodies that block the binding of IL-2 to isolated p75 chains (5,6,7,8). Binding studies with cells expressing only one of the chains also show no high affinity binding, and Scatchard analysis of these binding data is consistent with single homogeneous populations of receptors (9,10,5). Fusion of cell membranes from cell lines expressing only p55 chains with a cell line expressing only p75 chains gives rise to hybrid membranes that exhibit high affinity binding (11). Transfection of the gene coding for the p75 chain into a Jurkat transformed clone, expressing only the p55 chain and showing only low affinity IL-2 binding, resulted in the isolation of new clones that expressed both p55 and p75 chains, and which showed high affinity IL-2 binding (5). High affinity binding is thus presumed to be due to the formation of p55-p75 heterodimers. Kinetic studies reinforce this notion. Measurements of the rate of IL-2 binding and dissociation from cells expressing only p55 chains, only p75 chains, or both, established that the high affinity receptor had a rapid on rate constant, characteristic of the p55 chain and a slow off rate constant, characteristic of the p75 chain (3).

Although the existence of the high affinity heterodimer is no longer in question, certain aspects about its formation are. In particular, it is still being debated whether the heterodimer can exist in the absence of IL-2, or whether it requires the presence of IL-2 for its formation. Chemical crosslinking studies indicate that the heterodimer exists in the absence of IL-2 (12,13), but Honjo and his collaborators (14,15), based on a variety of kinetic binding studies, have proposed an “affinity conversion model” where heterodimers only form in the presence of IL-2. In their model heterodimers form by a two step process. First, IL-2 binds to the p55 chain with low affinity and then the IL-2-p55 complex interacts with a free p75 chain to form a heterodimer with IL-2 bound. This latter interaction causes the IL-2 to
become more tightly bound, creating the observed high affinity receptor. Recent studies using anti-p55 or anti-p75 antibodies to inhibit high affinity binding at 4 °C and 37 °C have been interpreted to support the affinity conversion model (16,3).

Many of the conclusions drawn from IL-2 binding studies have been based on interpretations of Scatchard plots. However, for systems involving different populations of interacting receptors the interpretation of Scatchard plots is by no means obvious. To obtain an interpretation of the initial slope of such a plot, or the x-intercept of the initial straight line portion of the plot extrapolated to the x-axis, or almost any other feature of such plots requires first specifying the interactions that can occur among the ligand and receptor populations in the form of a mathematical model. From this model analytic expressions can be derived for the features of the Scatchard plot that are of interest (e.g., initial slope, final slope, etc.). In this paper we carry out this procedure and develop a general model for IL-2 interactions with its various binding proteins. We then use this model to obtain rigorous interpretations for the slopes and intercepts of Scatchard plots obtained from IL-2 equilibrium binding experiments. Two special cases of this model correspond to the affinity conversion model, where there are no p55-p75 complexes in the absence of IL-2, and the preformed heterodimer model, where most of the p75 chains are complexed with p55 chains in the absence of IL-2.

We show that the affinity conversion model and the preformed heterodimer model make very different predictions about how Scatchard plots depend on the cell surface concentrations of the p55 and p75 chains. The results we derive (which are for equilibrium binding experiments and strictly hold only when synthesis, internalization and recycling are blocked) demonstrate that from binding studies on cell lines expressing different numbers of p55 chains, these models can be distinguished. We show that published IL-2 binding studies are consistent with a large concentration of preformed heterodimers being present on the cell surface and inconsistent with the affinity conversion model in which preformed heterodimers are excluded from the cell surface.
THE INTERACTIONS AMONG IL-2 AND ITS RECEPTORS

We consider an equilibrium model in which p55 and p75 chains can reversibly interact to form heterodimers in the absence of IL-2. We then focus on two limiting cases, one in which the equilibrium constant for this reaction is sufficiently small so that essentially no p55-p75 complexes are on the surface in the absence of IL-2 (the affinity conversion model), and one in which the equilibrium constant is sufficiently large so that most of the limiting chains (p75) are in heterodimers (the preformed heterodimer model).

In the presence of heterodimers there are at least three direct binding reactions that can occur: IL-2 can bind from solution to a free light chain (p55), to a free heavy chain (p75), or to a free dimer (p55-p75 heterodimer) on the surface of a cell. In describing these reactions we let $L$, $H$ and $D$ be the concentrations of free p55 chain, free p75 chain, and free p55-p75 heterodimer, and $L^*$, $H^*$ and $D^*$ be the concentrations of bound (complexed with IL-2) p55 chain, bound p75 chain and bound p55-p75 heterodimer, respectively. We let $C$ be the concentration of free IL-2 in solution. Schematically the direct binding reactions can be represented as follows:

\[
\begin{align*}
C + L \xrightleftharpoons[k_{L-}]{k_{L+}} L^* \\
C + H \xrightleftharpoons[k_{H-}]{k_{H+}} H^* \\
C + D \xrightleftharpoons[k_{D-}]{k_{D+}} D^*
\end{align*}
\]

(1a)

(1b)

(1c)

where the forward and reverse rate constants are indicated for each reaction. The equilibrium constants for reactions (1a), (1b) and (1c) are $K_L = k_{L+}/k_{L-}$ (low affinity), $K_H = k_{H+}/k_{H-}$ (intermediate affinity) and $K_D = k_{D+}/k_{D-}$ (high affinity). Since these equilibrium constants characterize the binding of a ligand that is free in solution they must have units of $1/(\text{solution concentration})$, e.g. M$^{-1}$.

Assuming all receptors, including the heterodimer, are monovalent for IL-2, the above reactions are the only direct binding reactions that can occur. In the affinity
conversion model reaction (1c) does not occur because heterodimers do not exist unless bound to IL-2.

When p55 and p75 chains are expressed on the same cell surface, they interact to form heterodimers. Three possible bimolecular reactions that can lead to heterodimer formation are:

\[ L + H \xrightarrow{k_{LH+}} D \]  
\[ L^* + H \xrightarrow{k_{L*H+}} D^* \]  
\[ L + H^* \xrightarrow{k_{LH^*+}} D^* \]

In the affinity conversion model reaction (2a) does not occur. Saito et al. (15) argue that on T cells that express considerably more light chain than heavy chain, the dominant reaction for forming the high affinity binding state is reaction (2b). The equilibrium constants for these reactions (2a), (2b) and (2c) are \( K_{LH} = \frac{k_{LH+}}{k_{LH-}} \), \( K_{L*H} = \frac{k_{L*H+}}{k_{L*H-}} \) and \( K_{LH^*} = \frac{k_{LH^*+}}{k_{LH^*-}} \). Since these equilibrium constants characterize bimolecular interactions among receptor chains on a cell surface they have units of \( 1/(\text{surface concentration}) \). We take all such equilibrium constants to have units of \( \text{cm}^2 \) and measure receptor concentrations in receptors/\( \text{cm}^2 \) by dividing the number of receptors per cell by the surface area of the cell. In sample calculations we take the cell surface area \( A = 5 \times 10^{-6} \text{ cm}^2 \), which corresponds to a smooth spherical cell of radius somewhat greater than 6 \( \mu \text{m} \), a reasonable size for an activated lymphocyte.

A possible additional bimolecular reaction is,

\[ L^* + H^* \rightleftharpoons D^{**} \]  

where \( D^{**} \) indicates a heterodimer with two IL-2 molecules bound. We assume this reaction does not occur, although we know of no convincing evidence either for or
against this assumption. However, if reaction (2d) does occur then the heterodimer must have a valence for IL-2 of two or greater. This would mean that there is at least one additional direct binding reaction that can occur, the binding of a second IL-2 molecule to a heterodimer with one IL-2 already bound. If reaction (2d) cannot occur, so that a bound p55 chain and a bound p75 chain cannot interact to form a heterodimer, then in the limit of very high IL-2 concentrations the number of high affinity receptors will go to zero and only bound p55 and bound p75 chains will be present on the cell surface.

There is a third type of reaction that may occur on the cell surface, namely a bound single chain may interact with a free heterodimer and transfer a bound IL-2 to the heterodimer. Again, in the affinity conversion model such reactions cannot occur because there are no free heterodimers, but there is no experimental evidence to rule out such reactions. Thus, the following transfer reactions will also be considered

\[
L^* + D \xrightleftharpoons[k_{L^*D^-}]{k_{L^*D^+}} L + D^* \quad (3a)
\]

\[
H^* + D \xrightleftharpoons[k_{H^*D^-}]{k_{H^*D^+}} H + D^* \quad (3b)
\]

where the equilibrium constants for these reactions are \(K_{L^*D} = k_{L^*D^+}/k_{L^*D^-}\) and \(K_{H^*D} = k_{H^*D^+}/k_{H^*D^-}\). These equilibrium constants are dimensionless since they refer to reactions were two receptors interact to form two other receptors.

Although eight equilibrium constants have been introduced in describing reactions (1) – (3), we shall see in the next section that these are not all independent. In fact, only four equilibrium constants are required to characterize the eight reactions.

FORMULATING THE MATHEMATICAL MODEL

At equilibrium (i.e., at long times with internalization, recycling and synthesis blocked) the following relationships hold among the ligand and receptor concentrations. From the direct binding reactions, (1a), (1b) and (1c), we have that

\[
L^* = K_L CL \quad (4a)
\]
\[ H^* = K_H CH \]  \hspace{1cm} (4b)  
\[ D^* = K_D CD \]  \hspace{1cm} (4c)

From the bimolecular reactions, (2a), (2b) and (2c), we have that at equilibrium

\[ D = K_{LH} LH \]  \hspace{1cm} (5a)  
\[ D^* = K_{L*H} L^*H \]  \hspace{1cm} (5b)  
\[ D^* = K_{LH*} LH^* \]  \hspace{1cm} (5c)

From the transfer reactions, (3a) and (3b), we have that

\[ D^* = K_{L*DL^*D/L} \]  \hspace{1cm} (6a)  
\[ D^* = K_{H*DH^*D/H} \]  \hspace{1cm} (6b)

By equating different expressions for \( D^* \), Eq (4c), (5b), (5c), (6a) and (6b), and substituting appropriately, the following relations among the various equilibrium constants can be obtained. These are known as detailed balance constraints (17).

\[ K_{L*H} = K_D K_{LH}/K_L \]  \hspace{1cm} (7a)  
\[ K_{LH*} = K_{L*H} K_L/K_H \]  \hspace{1cm} (7b)  
\[ K_{H*D} = K_D/K_H \]  \hspace{1cm} (7c)  
\[ K_{L*D} = K_{H*D} K_K/K_L \]  \hspace{1cm} (7d)

In the affinity conversion model only four reactions are allowed, (1a), (1b), (2b) and (2c), and therefore only Eqs. (4a), (4b), (5b) and (5c) apply to this model. Of the four equilibrium constants in these equations only three are independent. The fourth equilibrium constant can be calculated from Eq. (7b), which holds for the affinity conversion model and the preformed heterodimer model.

These constraints must be kept in mind when comparing the two models. Saito et al. (15) have argued that reaction (2b) is the dominant pathway for forming heterodimers and therefore that \( K_{L*H} \) must be large. It is important to realize that if there is any interaction between p55 and p75 chains in the absence of IL-2, even if such interactions are weak and lead to few heterodimers being formed,
then Eq. (7a) holds and $K_{LH} = K_{L^*H}K_L/K_D$. Thus the equilibrium constant for formation of dimer in the absence of IL-2 cannot be arbitrarily small, but is determined by $K_{L^*H}$ and the equilibrium constants for direct binding to the high and low affinity receptors, $K_D$ and $K_L$.

In addition to the equilibrium relationships there are two conservation laws that are needed to fully describe equilibrium binding. If $L_T$ and $H_T$ are the total (both in and out of heterodimers) equilibrium concentrations of light and heavy chains on the cell surface respectively, then

$$L_T = L + L^* + D + D^*$$  \hspace{1cm} (8a)

$$H_T = H + H^* + D + D^*$$  \hspace{1cm} (8b)

where for the affinity conversion model $D = 0$. It is useful to introduce the following definition as well

$$D_T(C) = D + D^*$$  \hspace{1cm} (9)

where $D_T(C)$ is the total concentration of heterodimers, both free and bound, at equilibrium when the free IL-2 concentration is C. We call the concentration of heterodimers in the absence of IL-2 $D_0$, i.e., $D_T(0) = D(0) = D_0$. We can calculate $D_0$ from Eq. (5a). In the absence of IL-2, $L^* = H^* = D^* = 0$ and Eq (5a) becomes $D_0 = K_{LH}(L_T - D_0)(H_T - D_0)$. When expanded this is a quadratic equation in $D_0$ with solution

$$D_0 = \frac{1 + K_{LH}R_T - \sqrt{(1 + K_{LH}R_T)^2 - 4K_{LH}^2L_TH_T}}{2K_{LH}}$$  \hspace{1cm} (10a)

where

$$R_T = L_T + H_T$$  \hspace{1cm} (10b)

(There are two solutions to this quadratic equation. We have chosen the physically meaningful solution, with the minus sign in front of the square root, which vanishes when one of the chain concentrations is set equal to zero.)

When $L_T \gg H_T$, as is the case for many of the experimental systems studied, Eq. (10a) can be approximated by

$$D_0 \approx \frac{K_{LH}L_T}{1 + K_{LH}L_T}H_T$$  \hspace{1cm} (10c)
Thus, when $L_T \gg H_T$, for there to be substantial heterodimer formation in the absence of IL-2 requires that $K_{LH}L_T \geq 1$. For example, when $K_{LH}L_T = 1$, Eq (10c) shows that approximately half of the heavy chains are incorporated into heterodimers in the absence of IL-2. If there are $10^4$ p55 chains on a cell with a surface area of $5 \times 10^{-6}$ cm$^2$, so that $L_T = 2 \times 10^9$ cm$^{-2}$, the inequality $K_{LH}L_T \geq 1$ is satisfied if $K_{LH} \geq 5 \times 10^{-10}$ cm$^2$.

To obtain expressions for Scatchard plots we need to express $B$, the total concentration of surface bound IL-2, as a function of the free IL-2 concentration, $C$ (recall that a Scatchard plot is a plot of $B/C$ versus $B$). The total concentration of surface bound IL-2

$$B = L^* + H^* + D^*$$

(11)

To determine the concentrations of the bound receptors, we use the conservation equations, Eq. (8a) and (8b), to rewrite the direct binding relations, Eqs. (4a), (4b) and (4c) as follows:

$$L^* = \frac{K_L C(L_T - D_T(C))}{1 + K_L C}$$

(12a)

$$H^* = \frac{K_H C(H_T - D_T(C))}{1 + K_H C}$$

(12b)

$$D^* = \frac{K_D C D_T(C)}{1 + K_D C}$$

(12c)

To obtain an expression for $D_T(C)$ in terms of $C$ and the total concentrations of light and heavy chains, $L_T$ and $H_T$, we note that from Eqs (4c) and (5a) that $D^* = K_D C D = K_D C K_{LH} L H$. Using the conservation laws, Eq. (8a) and (8b), to eliminate $L$ and $H$ this expression becomes $D^* = K_D C K_{LH} (L_T - L^* - D_T(C))(H_T - H^* - D_T(C))$. Finally, using Eqs (12a), (12b) and (12c) to eliminate $L^*$, $H^*$ and $D^*$ we obtain

$$D_T(C) = \frac{1 + K_D C}{(1 + K_L C)(1 + K_H C)} K_{LH} (L_T - D_T(C))(H_T - D_T(C))$$

(13)

This is a quadratic equation for $D_T(C)$ with solution

$$D_T(C) = \frac{1 + F(C) R_T - \sqrt{(1 + F(C) R_T)^2 - 4 (F(C))^2 L_T H_T}}{2 F(C)}$$

(14a)
where
\[
F(C) = \frac{(1 + K_D C) K_{LH}}{(1 + K_L C)(1 + K_H C)} = \frac{(K_{LH} + CK_L H K_L)}{(1 + K_L C)(1 + K_H C)} \tag{14b}
\]

The second form of \( F(C) \) is obtained from the detailed balance constraint Eq. (7a). Note that when \( C = 0 \), \( F(0) = K_{LH} \) and Eq. (14a) reduces to Eq. (10a). Note also that when \( K_{LH} = 0 \), which is the affinity conversion model, \( F(C) \) reduces to the expression obtained by setting \( K_{LH} = 0 \) in the last term of Eq. (14b). The concentration of heterodimers in the affinity conversion model can then be obtained by using this expression for \( F(C) \) in Eq. (14a).

We can calculate \( B \), the equilibrium concentration of bound IL-2, for any free IL-2 concentration by first calculating the total heterodimer concentration from Eq. (14a) and then calculating the concentrations of bound receptors from Eqs. (12a), (12b) and (12c). We can therefore predict the shape of a Scatchard plot if we know the total concentrations of light and heavy chain, \( L_T \) and \( H_T \), and four equilibrium constants: \( K_L \), \( K_H \), \( K_D \) and \( K_{LH} \). In the affinity conversion model it can be shown that only three equilibrium binding constants are needed: \( K_L \), \( K_H \) and \( K_{L-H} \).

**INTERPRETATION OF SCATCHARD PLOTS**

Scatchard plots have been used to draw qualitative and quantitative conclusions about the interactions of IL-2 with its receptors on T cells. In a typical IL-2 binding experiment at 37 °C, radioactively labeled IL-2 is allowed to bind to cells for 10-30 min and then the total amount of IL-2 associated with the cell is determined. The choice of the time for binding is made to maximize the chance of achieving equilibrium while minimizing the amount of IL-2 that is internalized. (For a human T cell line the half-life for internalization of bound high affinity receptors is about 15 min (18)). Because the system may not be in equilibrium at 10-30 min we call the resulting plots dynamic Scatchard plots (19). To analyze the data, \( B_T / C \) versus \( B_T \) is plotted, where \( B_T \) is the total cell associated IL-2, surface bound plus internalized, and \( C \) is the free IL-2 concentration. Four quantities are usually estimated from such plots, the equilibrium constant for binding of IL-2 to the heterodimer (from the initial slope), the concentration of the heterodimer (from the x-intercept of the initial straight line portion of the curve extrapolated to the x-axis), the equilibrium constant for binding to the low affinity p55 receptor (from the final slope), and
the concentration of p55 receptors (from the x-intercept of the final straight line portion of the curve.) Whether, under the conditions of these binding experiments, the initial slope, extrapolated x-intercept, final slope and final intercept have the meanings attributed to them is a difficult question to answer. Here we look at the simpler question, the interpretation of equilibrium Scatchard plots for the IL-2 system. Such plots are attained in the ideal situation where the total concentrations of light and heavy chains are constant (no internalization or insertion of receptors) and all chemical reactions have reached equilibrium.

Fig. 1 is a schematic of an equilibrium Scatchard plot, \( B/C \) versus \( B \), where \( B \) is the concentration of surface bound IL-2 and \( C \) is the free IL-2 concentration. Indicated in the figure are the following characteristics of the plot: the initial \((C \to 0)\) and final \((C \to \infty)\) slopes, \( S_0 \) and \( S_\infty \), the y-intercept, \( Y_{int} \), the initial x-intercept, i.e., the x-intercept obtained by extrapolating the initial straight line portion of the curve to the x-axis, \( X_0 \), and the true x-intercept obtained when all binding sites are filled, \( X_\infty \). If heterodimers cannot form from bound heavy and bound light chains, as we have assumed by ignoring reaction (2d), then \( X_\infty = R_T = L_T + H_T \).

Results For The Preformed Heterodimer Model

Recall that in the absence of IL-2 \((C = 0)\) the concentration of free heterodimers is \( D_0 \). It is useful to define \( L_0 \) and \( H_0 \) to be the concentrations of light and heavy chains that are not in heterodimers when \( C = 0 \) and \( R_0 \) to be the total number of receptors on the cell surface when \( C = 0 \). Thus,

\[
L_0 = L_T - D_0 \quad (15a)
\]

\[
H_0 = H_T - D_0 \quad (15b)
\]

\[
R_0 = L_0 + H_0 + D_0 = L_T + H_T - D_0 \quad (15c)
\]

We define the average affinity, \(<K>\), and the average squared affinity, \(<K^2>\), to be

\[
<K> = (L_0 KL + H_0 KH + D_0 KD)/R_0 \quad (16a)
\]

\[
<K^2> = (L_0 K_L^2 + H_0 K_H^2 + D_0 K_D^2)/R_0 \quad (16b)
\]
We also define

\[ a_1 = <K > R_0 \]  
\[ a_2 = < K^2 > R_0 - \frac{K_{LH} L_0 H_0}{1 + K_{LH}(L_0 + H_0)} (K_D - K_L - K_H)^2 \]

In terms of these quantities, we show in the Appendix that

\[ Y_{int} = a_1 \]  
\[ X_0 = a_1^2 / a_2 \]  
\[ S_0 = -a_2 / a_1 \]

One can also show that

\[ S_\infty = -R_T \left[ \frac{L_T}{K_L} + \frac{H_T}{K_H} + \frac{K_D}{K_L K_H} K_{LH} L_T H_T \right]^{-1} \]

Note that in the complete absence of heterodimer formation, \( D_0 = 0 \) and \( K_{LH} = 0 \), we recover the known results for a population of two noninteracting receptors, that \( Y_{int} = <K > R_T \), \( X_0 = R_T <K^2 > / <K^2 > \), \( S_0 = -<K^2 > / <K > \) and \( S_\infty = -1/ <K^{-1}> \), where the brackets stand for averages with respect to the receptor populations (20). If the two populations consist of a small population of high affinity receptors and a large population of low affinity receptors with equilibrium constants \( K_{high} \) and \( K_{low} \) respectively, then the expressions for the slopes reduce to \( S_0 \approx -K_{high} \) and \( S_\infty \approx -K_{low} \). However, if the receptor populations interact to form heterodimers that also bind the ligand this is no longer true as can be seen from Eqs. (18c) and (18d). For example, if the additional term due to aggregation in Eq. (18d) is large, the final slope \( S_\infty \) will be smaller than \( K_L \).

For most T cells and cell lines that express both p55 and p75 chains, the number of heavy chains is much smaller than the number of light chains. Thus, let us consider the case where (1) \( H_T / L_T \ll 1 \); and (2) in the absence of IL-2 almost all the heavy chains are in heterodimers. As can be seen from Eq. (10c), conditions (1) and (2) require that \( K_{LH} L_T \gg 1 \). If almost all the heavy chains are in heterodimers then \( H_0 \approx 0 \), \( D_0 \approx H_T \) and \( L_0 \approx L_T - H_T \). When this is so

\[ S_0 \approx -K_D \frac{1 + (K_L/K_D)^2((L_T/H_T) - 1)}{1 + (K_L/K_D)((L_T/H_T) - 1)} \]

14
For cells expressing both p55 and p75 chains the number of p75 chains is commonly estimated by assuming that \( H_T \approx X_0 \), and the affinity of the heterodimer is estimated by assuming that \( K_D \approx -S_0 \). From our analysis we see that this interpretation is correct at equilibrium, i.e., Eqs. (19a) and (19b) reduce to \( S_0 \approx -K_D \) and \( X_0 \approx H_T \), only if \( K_L/K_D \approx 1 \) and \( K_L L_T \ll K_D H_T \).

In summary, for cell lines with a preponderance of light chains, the preformed heterodimer model predicts that the initial slope of an equilibrium Scatchard plot will be approximately equal to the affinity of the heterodimer if (1) \( K_L H L_0 \gg 1 \), i.e., in the absence of IL-2 almost all the heavy chains are in heterodimers; (2) \( K_L/K_D \ll 1 \), i.e., the light chain has a much lower affinity than the heterodimer; and (3) \( K_L L_T \ll K_D H_T \). We have pointed out that for a cell with \( 10^4 \) light chains on its surface condition (1) is satisfied if \( K_L H \gg 5 \times 10^{-10} \text{ cm}^2 \). Since the heterodimer is the high affinity receptor and the light chain is the low affinity receptor, condition (2) is always satisfied. Wang and Smith (3) carried out equilibrium binding experiments on a T cell line (MT-1) expressing only light chains and found \( K_L = 7.1 \times 10^7 \text{ M}^{-1} \). Thus, on a T cell expressing \( 10^4 \) light chains and \( 2 \times 10^3 \) heavy chains (3) condition (3) is satisfied if \( K_D \gg 3.6 \times 10^8 \text{ M}^{-1} \). Initial slopes of dynamic Scatchard plots at 37 °C and equilibrium Scatchard plots at 0 °C suggest \( K_D \approx 5 \times 10^{10} - 5 \times 10^{11} \text{ M}^{-1} \) (21,4,22,3,11,5). Thus, for our model T cell condition (3) is satisfied.

We illustrate the predictions of the preformed heterodimer model for a cell with \( 2 \times 10^3 \) heavy chains and \( 1 \times 10^4 \) light chains on its surface. We take \( K_L = 7.1 \times 10^7 \text{ M}^{-1} \) and \( K_H = 8.3 \times 10^8 \text{ M}^{-1} \). These values were determined by Wang and Smith (3) from equilibrium binding studies at 37 °C using cells expressing only light or heavy chains. \( K_H \) was determined from membrane binding studies to insure that there was no internalization or synthesis. These values for \( K_L \) and \( K_H \) are consistent with a number of other binding studies (22,11,10,5). We take \( K_D = 1 \times 10^{11} \text{ M}^{-1} \) although this affinity has not been directly determined. (Wang and Smith (3) estimated that \( K_D = 7.7 \times 10^{10} \text{ M}^{-1} \) from binding studies at 37 °C on cells expressing both light and heavy chains. In order to determine \( K_D \) they assumed that the initial slope of their 20 min Scatchard plots, with neither internalization nor

\[
X_0 \approx H_T \frac{[(1 + (K_L/K_D)((L_T/H_T) - 1))]^2}{1 + ((K_L/K_D)^2((L_T/H_T) - 1))}
\]
synthesis blocked, equaled $K_D$. Their kinetic measurements were in good agreement with this value, but since there are many possible kinetic pathways for forming a bound heterodimer, Eqs. (1a), (2b), (2c), (3a) and (3b), the forward and reverse rate constants that they determined may not equal $k_{D+}$ and $k_{D-}$. This would only be true if, for the IL-2 concentrations used in their kinetic studies, the dominant reaction for forming and dissociating from a bound heterodimer was the direct reaction. Equilibrium Scatchard plots at 0 °C give $K_D = 1.2 \times 10^{11} \text{ M}^{-1}$ which is consistent with estimates of $K_D$ from dynamic Scatchard plots (22). There has been no experimental estimate of $K_{LH}$ but, as we have discussed, if the initial slope of the Scatchard plot is to reflect direct binding to the high affinity heterodimer, a large fraction of the heavy chains must be in heterodimers. For a cell with $10^4$ light chains and a surface area of $5 \times 10^{-8} \text{ cm}^2$ this requires that $K_{LH} \gg 5 \times 10^{-10} \text{ cm}^2$. Shown in Fig. 2 is the model prediction of the effect of $K_{LH}$ on the initial slope of the Scatchard plot. As $K_{LH}$ is increased from $10^{-10} \text{ cm}^2$ to $10^{-8} \text{ cm}^2$, the initial slope varies from $-2.8 \times 10^{9} \text{ M}^{-1}$ to $-9.4 \times 10^{10} \text{ M}^{-1}$ while the number of preformed heterodimers, $D_0$, increase from $2.9 \times 10^3$ per cell (2 % of the heavy chains in heterodimers) to $1.9 \times 10^3$ per cell (95 % of the heavy chains in heterodimers). In the preformed heterodimer model, when the concentration of light chains is in excess, a large fraction of the heavy chains must be in heterodimers in the absence of IL-2 if the initial slope of the Scatchard plot is to be approximately equal to the affinity of the heterodimer.

In summary, when $L_T > H_T$, if a large fraction of heavy chains are in dimers in the absence of IL-2, the initial slope of the equilibrium Scatchard plot will be approximately equal to minus the affinity of the heterodimer. Under these conditions the initial slope will be insensitive to the concentration of light chains over a wide range light chain concentrations. Similar the initial x-intercept $X_0$ will give a reasonable estimate of the number of preformed heterodimers.
Results for the Affinity Conversion Model

We now consider the behavior of the Scatchard plot when the number of preformed heterodimers is negligible. This corresponds to letting $K_{LH}$ approach zero. Using the detailed balance constraint, Eq. (7a), $K_D = K_{L\cdot H}K_L/K_{LH}$, we eliminate $K_D$ from Eqs. (16) and (17). Taking the limit that $K_{LH} = 0$, we obtain the expressions below that characterize the affinity conversion model.

\begin{align*}
< K > &= (LTK_L + HTK_H + HTLTK_LK_{L\cdot H})/RT \quad (20a) \\
< K^2 > &= (LTK_L^2 + HTK_H^2 + HTLTRTK_L^2K_{L\cdot H}^2)/RT \quad (20b) \\
0_1 &= < K > RT \quad (21a) \\
0_2 &= < K^2 > RT + 2HTLTK_LK_{L\cdot H}(K_L + K_H) \quad (21b)
\end{align*}

(An alternate way to derive these expressions, which is presented in the Appendix, is to start by assuming that there are no free heterodimers, i.e., $D = 0$ for all $C$.) Using Eqs. (21a) and (21b) we can now calculate from Eqs. (18a), (18b) and (18c), $Y_{int}$, $X_0$ and $S_0$ (see Fig. 1) for a Scatchard plot as predicted by the affinity conversion model. One can show that if the initial slope of the Scatchard plot is to be much larger than both $K_L$ and $K_H$, then $HTLTK_LK_{L\cdot H} \gg LTK_L + HTK_H$, which can be rewritten as

\begin{equation}
K_{L\cdot H} \gg 1/HT + K_H/(K_LL_T)
\end{equation}

Under this condition

\begin{equation}
S_0 \approx -RTK_LK_{L\cdot H} = -K_{ac}
\end{equation}

where $K_{ac}$ is the apparent affinity of the high affinity receptor in the affinity conversion model. Further, we find that the apparent number of high affinity receptors, i.e., the initial $X$-intercept is given by

\begin{equation}
X_0 \approx LTHT/RT
\end{equation}

Note that when $L_T \gg HT$ from Eq. (23b), $X_0 \approx HT$. 17
From Eq. (23a) we see that the affinity conversion model predicts that the initial slope of the Scatchard plot is proportional to the total number of cell surface receptors. We shall look at this prediction in more detail, but first we estimate the value of $K_L \cdot H$, the equilibrium constant for the interaction of a bound light chain with a heavy chain to form a bound dimer. We again take $K_L = 7.1 \times 10^7 \text{ M}^{-1}$, $K_H = 8.3 \times 10^8 \text{ M}^{-1}$, and consider a cell with $2 \times 10^3$ heavy chains and $1 \times 10^4$ light chains ($R_T = 1.2 \times 10^4$ chains/cell or $2.4 \times 10^9$ chains/cm$^2$ for a cell with a surface area of $5 \times 10^{-6}$ cm$^2$). Eq. (23a) and (23b) will hold when Eq. (22) is satisfied, i.e., when $K_L \cdot H \gg 8 \times 10^{-9}$ cm$^2$. Eq. (23b) predicts that in the affinity conversion model, in order for an equilibrium Scatchard plot to have an initial slope of $1 \times 10^{11}$ M$^{-1}$, $K_L \cdot H = 6 \times 10^{-7}$ cm$^2$.

In summary, when Eq. (22) is satisfied, the affinity conversion model predicts that the slope of the equilibrium Scatchard plot will be given by Eq. (23a) and the intercept by Eq. (23b). Thus, when $L_T \gg H_T$, the initial slope $S_0$ is predicted to be directly proportional to the concentration of light chains on the cell surface.

**Comparison of The Two Models**

Equilibrium Scatchard plots at 0 °C and 4 °C and dynamic Scatchard plots at 37 °C for IL-2 binding experiments on cells expressing both light and heavy chains, for a variety of light and heavy chain concentrations, yield initial slopes ranging from approximately $-3 \times 10^{10}$ M$^{-1}$ to $-3 \times 10^{11}$ M$^{-1}$ (21,23,22,4,24,3). For cells having $2 \times 10^3$ heavy chains per cell we have chosen $K_L H$ and $K_D$ in the preformed heterodimer model, and $K_L \cdot H$ in the affinity conversion model, so that when there are $1 \times 10^4$ light chains per cell in both models the initial slope $S_0 = 1 \times 10^{11}$ M$^{-1}$. Shown in Fig. 3 are the initial portions of the predicted Scatchard plots for $L_0 = 10^3$, $10^4$ and $10^5$ chains per cell. One sees that $S_0$ is predicted to change little in the preformed heterodimer model, but to decrease dramatically as $L_0$ decreases in the affinity conversion model. While studying the regulation of p55 expression, Smith and Cantrell (23) measured the initial slopes of Scatchard plots for two T cell populations, one with $4.3 \times 10^4$ and one with $1.0 \times 10^4$ p55 chains (determined by binding of anti-Tac antibody) and found the initial slopes to be essentially the same ($K_D = 4.8 \times 10^{10}$ M$^{-1}$ and $4.4 \times 10^{10}$ M$^{-1}$). The affinity conversion model would predict a difference in slopes of approximately a factor of 4 for these experiments.
Robb and his collaborators (21,4,22) have determined the initial slope of dynamic Scatchard plots for a set of T cells exhibiting a wide range of light chain concentrations. These data are plotted in Fig. 4 along with fits of the slopes predicted by the preformed heterodimer model and the affinity conversion model. There is no observed decrease in the experimentally determined slopes as the total number of receptors decrease.

**DISCUSSION**

Two transmembrane IL-2 binding proteins have been identified, a p55 chain that binds IL-2 with low affinity \( (K_L \approx 10^8 \text{ M}^{-1}) \) and a p75 chain that binds IL-2 with intermediate affinity \( (K_H \approx 10^9 \text{ M}^{-1}) \). When both p55 and p75 chains are expressed on the same cell, Scatchard analysis indicates the presence of high affinity receptors \( (K_D \approx 10^{11} \text{ M}^{-1}) \). Two models have been proposed to explain this high affinity binding. In one, the preformed heterodimer model, p55-p75 heterodimers form in the absence of IL-2 and directly bind IL-2 with high affinity. In the other, the affinity conversion model, there are no preformed heterodimers, but rather high affinity heterodimers form through a two step process. First, IL-2 binds to a p55 chain and then the IL-2-p55 complex aggregates with a free p75 chain.

We have developed a general model for the equilibrium binding of IL-2 to its cell surface receptors. The preformed heterodimer model and the affinity conversion model are special cases of this model. A critical parameter in the model is \( K_{LH} \), the equilibrium constant for the formation of heterodimers from p55 and p75 chains in the absence of IL-2. On cells where the number of p55 chains greatly exceeds the number of p75 chains a large value of \( K_{LH} (K_{LH} \geq 10^{-8} \text{ cm}^2) \) implies that almost all the p75 chains will be in heterodimers in the absence of IL-2 (the preformed heterodimer model), while a small value of \( K_{LH} (K_{LH} \leq 10^{-10} \text{ cm}^2) \) implies that essentially no p75 chains will be in preformed heterodimers (the affinity conversion model). We have used the general model to predict the characteristics of Scatchard plots for the equilibrium binding of IL-2 to its surface receptors. A striking difference between the preformed heterodimer model and the affinity conversion model is the predicted dependence of the initial slope of an equilibrium Scatchard plot on p55 and p75 chain concentrations. When (1) the magnitude of the initial slope is
much larger than the affinities of IL-2 for isolated p55 and p75 chains (which is what is observed); and (2) the concentration of p55 chains is much larger than p75 chains; the preformed heterodimer model predicts that the slope will be independent of the chain concentrations and equal to the affinity of IL-2 for the heterodimer. The preformed heterodimer model further predicts that in the absence of IL-2 almost all the p75 chains will be in heterodimers. For the same conditions the affinity conversion model predicts that the initial slope of the Scatchard plot will increase linearly with the total chain concentration. This difference arises from the different mechanisms for the formation of high affinity receptors. In the preformed heterodimer model high affinity receptors pre-exist and are assumed to have an affinity $K_D \approx 10^{11} \text{ M}^{-1}$ for IL-2. The slope of the Scatchard plot is predicted to approximately equal this affinity when a large fraction of the p75 chains are in preformed heterodimers. Once the p55 chain concentration is high enough so that most of the p75 chains are in heterodimers, further increasing the number of p55 chains has little effect on the number of heterodimers. In the affinity conversion model there are no preformed high affinity receptors and hence the initial slope of the Scatchard plot reflects the various interactions needed to form the high affinity p55-IL-2-p75 complex. Analysis shows that the initial slope of the Scatchard plot is given by the expression $-K_L K_{L \cdot H} (L_T + H_T)$, where $K_L$ is the equilibrium constant for binding of an IL-2 to a p55 chain, $K_{L \cdot H}$ is the equilibrium constant for binding of an IL-2-p55 complex to a p75 chain, and $L_T$ and $H_T$ are the total concentrations of p55 and p75 chains, respectively. Thus, for $L_T \gg H_T$ the slope in the affinity conversion model is predicted to be proportional to the number of p55 chains on the cell surface.

From published experiments of Robb and his collaborators (21,4,22) and Smith and Cantrell (23) we conclude that the affinity conversion model, where there are no preformed dimers, is inconsistent with these data. For a wide range of total chain concentrations ($10^3 - 10^5$ chains/cell) the initial slope of a Scatchard plot for the binding of IL-2 to receptors on T cells remains unchanged. This observation is consistent with the preformed heterodimer model. There is however one caveat. The model predictions are for equilibrium Scatchard plots. The experiments we cite were done at 37 °C without blocking internalization, recycling or protein synthesis. How well Scatchard plots for such binding data approximate equilibrium Scatchard plots
is unknown, although predictions of $K_D$ from such plots are consistent with measurements at 0 °C (22). The second prediction of the preformed heterodimer model, that a large fraction of the p75 chains are in preformed heterodimers, has yet to be tested, although there are indications that preformed heterodimers exist (12,13). Since the model presented is an equilibrium model it makes no predictions about the stability of preformed heterodimers. Indeed, since it is an equilibrium model, it assumes that all reactions are reversible, so that at equilibrium heterodimers are constantly forming and breaking apart.

We considered eight distinct chemical interactions and introduced eight equilibrium constants to characterize these interactions. However, we have shown that only four of these equilibrium constants are needed to fully describe the binding. This is due to the thermodynamic principle that the free energy of a ligand-receptor complex is independent of the way the complex was formed. Thus the concentration of bound heterodimer at equilibrium can be calculated assuming it was formed by an IL-2 interacting with a free heterodimer, Eq (4c), or by a bound light chain interacting with a free heavy chain, Eq. (5b), or a combination of these pathways. If cell bound heterodimers are equivalent then there are only four independent equilibrium constants. This would mean for example, that equilibrium binding experiments cannot be used to ascertain whether transfer reactions, Eq. (3a) and (3b), occur since the equilibrium constants for these reactions are not needed to determine any of the equilibrium concentrations. However, if different pathways of bound heterodimer formation lead to states with different free energies of binding, e.g. phosphorylated or unphosphorylated receptors with different affinities for IL-2, then these different heterodimer states would need to be distinguished in the model and additional equilibrium constants would be required to describe the binding. As yet no such additional states have been found. Indeed, it has been shown that activation of protein kinase C, which is known to lead to the phosphorylation of p55 chains, does not affect the binding properties of the heterodimer as assessed from Scatchard analysis (22).

A main feature of the affinity conversion model (25,14,15), that is not addressed in this paper, is the kinetic mechanism proposed for the formation of the bound heterodimer. Honjo and his collaborators argue that the dominant mechanism for
such formation is first, the binding of IL-2 to the p55 chain, reaction (1a), and then
the interaction of the IL-2-p55 chain complex with a p75 chain, reaction (2b). The
work presented in this paper applies only to equilibrium experiments and makes no
comments about kinetic mechanisms, but since our analysis indicates that preformed
heterodimers exist, both direct binding to preformed heterodimers, reaction (1c),
and transfer reactions involving heterodimers, reaction (3a) and (3b), cannot be
ruled out as important kinetic mechanisms.

To study the roles of p55 and p75 chains in the formation of high affinity IL-2
receptor complex, experiments have been performed using anti-p55 and anti-p75
monoclonal antibodies (16,8). For example, Kamio et al. (16) were able to block
IL-2 high affinity binding at 4 °C with an anti-p75 antibody, 2R-B, but at 37 °C
IL-2 displaced 2R-B. They also showed that in the presence of anti-p55 antibody,
which inhibited IL-2 binding to p55, IL-2 was less effective in displacing 2R-B.
Kamino et al. (16) conclude that IL-2 bound to p55 was responsible for displacing
2R-B and therefore that the IL-2-p55 complex plays a key role in the formation
of the high affinity IL-2 receptor. These experiments are not inconsistent with the
preformed heterodimer model if one assumes that the binding of the 2R-B antibody
to p75 chains interferes with their association with p55 chains in the absence of IL-2.
This would lower the concentration of preformed heterodimers and make the system
behave as if preformed heterodimers were not present. Similarly, we believe that the
experiments of Audrain et al. (8) are compatible with the preformed heterodimer
model if one assumes that their anti-p75 antibody, TU27, binds isolated p75 binds
well, but p75-p55 and p75-IL-2-p55 complexes poorly or not at all.

The model we have presented applies to all receptor systems composed of two chains
which bind the ligand with different affinities and which also aggregate to form a
third affinity state. The first example of such a receptor system was the p55 and
p75 IL-2 binding chains. It now appears that the granulocyte-macrophage colony-
stimulating factor receptor system is a second example (26).

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APPENDIX

A. Scatchard Plots

We follow the derivation of Wofsy and Goldstein (unpublished results). The amount of ligand bound to receptors, $B$, is a function of the free ligand concentration, $C$. When $C$ is small we can expand $B$ in a power series in $C$, i.e., $B = a_1 C - a_2 C^2 + \cdots$, so that

$$\frac{B}{C} = a_1 - a_2 C + \cdots$$  \hspace{1cm} (A1)

For convenience we introduce a minus sign in the second term of the expansion. Doing this makes no assumptions about the binding since we allow $a_2$ to be positive or negative. If we keep only the first two terms in the expansion then

$$\frac{B}{C} \approx a_1 [1 - (a_2/a_1)C] \approx \frac{a_1}{1 + (a_2/a_1)C}$$  \hspace{1cm} (A2)

The last two terms in Eq. (A2) are equal when terms higher than the second in the expansion are neglected. Cross-multiplying the first and last terms in Eq. (A2) we have that

$$\frac{B}{C} \approx -(a_2/a_1)B + a_1$$  \hspace{1cm} (A3)

which is exact in the limit that $C \to 0$. From this equation, Eqs. (18a), (18b) and (18c) follow.

B. The Affinity Conversion Model

From the direct binding reactions (4a) and (4b), and the conservation laws, Eqs. (8a) and (8b), it follows that for the affinity conversion model $(D = 0)$:

$$L^* = \frac{K_L C}{(1 + K_L C)} (L_T - D^*)$$  \hspace{1cm} (B1)

$$H^* = \frac{K_H C}{(1 + K_H C)} (H_T - D^*)$$  \hspace{1cm} (B2)

These equations also follow from Eqs. (12a) and (12b) once it is recognized that $D_T(C) = D^*$ since $D = 0$. In the affinity conversion model heterodimers only exist when bound to IL-2. In the presence of IL-2, heterodimers can form in two
ways, by reactions (5b) and (5c). From Eqs. (5b) and Eq. (4c) it follows that
\[ D^* = K_L L^* H = CK_L L H. \]
Using the conservation laws, Eqs (8a) and (8b)
\[ D^* = CK_L L H (L_T - L^* - D^*) (H_T - H^* - D^*) \quad (B3) \]
Substituting Eqs. (B1) and (B2) to eliminate \( L^* \) and \( H^* \) in Eq. (B3)
\[ D^* = CK_L L H \frac{(L_T - D^*) (H_T - D^*)}{(1 + K_L C)(1 + K_H C)} \quad (B4) \]
which agrees with Eq. (14b) when \( K_{LH} = 0 \). Solving this equation for \( D^* \) yields
\[ D^*(C) = \frac{1 + F(C)R_T - \sqrt{(1 + F(C)R_T)^2 - 4(F(C))^2 L_T H_T}}{2F(C)} \quad (B5) \]
where
\[ F(C) = \frac{CK_L L H}{(1 + K_L C)(1 + K_H C)} \quad (B6) \]
For the affinity conversion model we can now calculate the equilibrium concentration
of any type of receptor for any free IL-2 concentration. From Eq. (B5) we can
calculate \( D^* \). Knowing \( D^* \) we can calculate \( L^* \) and \( H^* \) from Eqs. (B1) and (B2).
From the conservation laws, Eqs. (8a) and (8b) we can then calculate \( L \) and \( H \).

To obtain expressions for \( S_0 \) and \( X_0 \) (see Fig. 1) we must obtain the first two
coefficients in the power series expansion in \( C \) of \( B/C \). From Eqs. (B1) and (B4)
\[ B/C = \frac{K_L}{(1 + K_L C)}(L_T - D^*) + \frac{K_H}{(1 + K_H C)}(H_T - D^*) + \\
CK_L L H \frac{(L_T - D^*) (H_T - D^*)}{(1 + K_L C)(1 + K_H C)} \quad (B7) \]
where \( D^* \) is given by Eq. (B5). When this expansion is preformed one obtains
\[ a_1 = L_T K_L + H_T K_H + L_T H_T K_L K_L^* H \\
a_2 = L_T K_L^2 + H_T K_H^2 + \\
L_T H_T (K_L K_L^* H)^2 (L_T + H_T) + 2L_T H_T K_L K_L^* H (K_L + K_H) \quad (B8) \]

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FIGURE CAPTIONS

Figure 1. An equilibrium Scatchard plot. \( B \) is the concentration of bound ligand and \( C \) is the concentration of free ligand. \( S_0 \) and \( S_\infty \) are the initial \((C \rightarrow 0)\) and final \((C \rightarrow \infty)\) slopes, \( X_0 \) is the initial intercept obtained by extrapolating the initial straight line portion of the curve to the x-axis \((B/C = 0)\), and \( X_\infty \) is the final intercept. In the absence of receptor aggregation \( X_\infty \) always equals the total number of available receptor binding sites.

Figure 2. The effect of preformed heterodimers on the initial slope of a Scatchard plot as predicted by the preformed heterodimer model. \( K_{LH} \) is the equilibrium constant for the formation of a heterodimer from an unbound p55 chain and an unbound p75 chain, reaction (2a). The insert shows how the number of heterodimers on the cell surface in the absence of IL-2, \( D_0 \), is predicted to vary with \( K_{LH} \). The calculations are for a model cell characterized by the following parameters: \( L_T = 1 \times 10^4 \) p55 chains/cell, \( H_T = 2 \times 10^3 \) p75 chains/cell, \( K_L = 7.1 \times 10^7 \) M\(^{-1}\), \( K_H = 8.3 \times 10^8 \) M\(^{-1}\), and \( K_D = 1 \times 10^{11} \) M\(^{-1}\). Receptor concentrations were converted from receptors/cell to receptors/cm\(^2\) by taking the surface area of the cell \( A = 5 \times 10^{-6} \) cm\(^2\). The bound IL-2 concentration is the sum of the bound p55 chains, p75 chains and heterodimer concentrations, i.e., \( B = L^* + H^* + D^* \). \( B \) was calculated as follows: for each value of \( C \), the total heterodimer concentration, \( D_T(C) \), was obtained from Eq. (14a). Knowing \( D_T(C) \), the concentrations of bound p55 chains, p75 chains and heterodimers, \( L^* \), \( H^* \) and \( D^* \), were calculated from Eqs. (12a), (12b) and (12c).

Figure 3. Scatchard plots for three p55 chain concentrations concentrations, \( L_0 = 10^3 \), \( 10^4 \) and \( 10^5 \) chains/cell, as predicted by a) the affinity conversion model and b) the preformed heterodimer model. The calculations are for a model cell characterized by the following parameters: \( H_T = 2 \times 10^3 \) p75 chains/cell, \( K_L = 7.1 \times 10^7 \) M\(^{-1}\) and \( K_H = 8.3 \times 10^8 \) M\(^{-1}\). a) In the affinity conversion model we took \( K_{L^*H} = 6 \times 10^{-7} \) cm\(^2\). b) In the preformed heterodimer model we took \( K_D = 1 \times 10^{11} \) M\(^{-1}\) and \( K_{LH} = 1 \times 10^{-8} \) cm\(^2\). With these parameters both models predict an initial Scatchard plot of approximately \( 1 \times 10^{11} \) when \( L_0 = 1 \times 10^4 \) p55 chains/cell.
Figure 4. Comparison of model predictions with published data. $S_0$ is the initial slope of a Scatchard plot and $R_T = H_T + L_T$ is the total concentration of p55 and p75 chains. The data is from 37 °C binding studies on T cells: HUT 102B2 (●), PHA blasts (■), PBL (□), MLC blasts (▲) (21,4,22). To determining $R_T$ from the data we assumed that $H_0 \approx X_0$ and that the total number of binding sites for anti-Tac antibody as determined from Scatchard plots equaled $L_0$. For all the data $L_0 \gg H_0$. For this case the preformed heterodimer model predicts that $S_0 \approx -K_D$. The solid line is the best fit to the data when we take $S_0 = -K_D$. (Approximating the exact slope in this way introduces an error of less than 2% for all data points except the point with the lowest $R_T$. This point was not used in the fit.) The affinity conversion model predicts that $S_0 \approx -R_T K_L K_L^\ast H$ when Eq. (22) holds. The dashed line is the best fit to the data when we take $S_0 = -R_T K_L K_L^\ast H$ (Approximating the exact slope in this way introduces an error of less than 5% for data points except the point with the lowest $R_T$. This point was not used in the fit.)
REFERENCES


Fig. 3

Bound (molec./cell) / Free(pM) vs. Bound (molec./cell)