Crystallization and Preliminary X-ray Diffraction Studies of a Complex between Interleukin-2 and a Soluble Form of the p55 Component of the High Affinity Interleukin-2 Receptor*

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The critical role played by interleukin-2 (IL-2)1 in the mediation of immune responses is widely documented (1). IL-2 is produced by T cells that have been activated by antigens or mitogens and binds to specific low and high affinity cell surface receptors on target cells. The amino acid sequences of both human (2, 3) and murine (4) IL-2 have been determined. Various glycosylated forms of both receptor and ligand can be cocrystallized under those conditions. The best crystals of the putative receptor-ligand complex involve the enzymatically desialylated receptor and unglycosylated IL-2. These crystals belong to the trigonal space group P3,21 or its enantiomorph, with unit cell dimensions a = b = 91 Å and c = 119 Å, and diffract to 3.5 Å resolution. There is one receptor-ligand complex asymmetric unit, with a Matthews coefficient of 2.7, assuming the presence of one IL-2 molecule-receptor molecule. Interestingly, in addition to IL-2 (Mr = 14,000), the p55 IL-2 receptor (Mr = 44,000) and two fragments of the receptor, of apparent Mr = 35,000 and 25,000, respectively, in sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the crystals are enriched in a reducible dimeric form of the desialylated receptor (apparent Mr = 90,000), as compared with protein solution from which the crystals grow. The overall amino acid content in the crystals is consistent with a 1:1 ratio of receptor to ligand. A native data set has been collected on a multicrystal area detector and the search for suitable heavy atom derivatives is in progress.

The critical role played by interleukin-2 (IL-2)1 in the mediation of immune responses is widely documented (1). IL-2 is produced by T cells that have been activated by antigens or mitogens and binds to specific low and high affinity cell surface receptors on target cells. The amino acid sequences of both human (2, 3) and murine (4) IL-2 have been determined. In the human, the nucleotide sequence codes for a protein of 153 amino acids, including a signal sequence of 20 residues. Human IL-2 contains a disulfide bridge between residues Cys-58 and Cys-105 (5-7); it is O-glycosylated at position Thr-3 and contains sialic acid (7, 8). Circular dichroism studies, computer-assisted predictive methods, and 3.0 Å crystallographic studies suggest a highly helical structure for human IL-2 (9, 10).

A cell surface receptor, specific for IL-2, was initially detected on activated T cells with the use of a monoclonal antibody (anti-Tac) directed against antigen- or mitogen-activated T cells; anti-Tac does not react with IL-2 but suppresses IL-2 dependent T cell proliferation (8, 11-13). The Tac antigen was identified as a membrane-bound specific IL-2 receptor with a molecular mass of 55,000-60,000 daltons and a pI of 5.5-6.0, and is now usually referred to as the p55 (α, light chain) IL-2 receptor (IL-2R). The dissociation constant for the binding of IL-2 to the p55 IL-2R lies in the 10⁻⁹ M range (14).

Extensive biochemical characterization of the human p55 IL-2R has been reported. It is a glycoprotein containing two N-linked and several O-linked oligosaccharide moieties (15). A 33,000-dalton precursor is post-translationally modified to become the 55,000-dalton mature form. The primary structure of the p55 IL-2 receptor has been deduced from the nucleotide sequences of cDNA clones from the human (16-18), the mouse (19, 20), and the ox (21). The mature protein contains 251 amino acids including an extracellular domain of 219 residues, a transmembrane region of 19 residues, and a short carboxyl-terminal cytoplasmic tail of 13 residues. There are 12 Cys residues, forming five intramolecular disulfide linkages (22, 23). Phosphorylation of the receptor has been demonstrated on the cytoplasmic portion of the molecule, at residue Ser-247 (24, 25). The protein contains sulfate (26) and sialic acid present on at least some of the O-linked sugars (15).

Recently, a second IL-2 receptor molecule has been described (14, 27-30). This protein, or family of proteins, has a Mr = 70,000-75,000 and binds IL-2 with a Kd of approximately 10⁻⁹ M. This receptor, referred to as the p70 (β or heavy chain) IL-2 receptor, is glycosylated and contains a longer cytoplasmic domain than p55 (31). It has also been demonstrated that the p70 IL-2R is required for biological activity and internalization of IL-2 (32).

The association of p55 and p70 is believed to form the functional high affinity IL-2 receptor, with a Kd of 10⁻¹¹ M. Different portions of the IL-2 molecule appear to bind to the two components (subunits) of the high affinity receptor (29). To date, however, little is known about the formation of the putative ternary complex and it is not clear in which order IL-2, p55, and p70 IL-2R molecules associate to form this biologically active complex. Only recently have emerged reports of the demonstration, by cross-linking experiments, of the existence of such a ternary complex (33, 34).

The stoichiometry of the different IL-2 receptor/ligand complexes has not yet been determined but has generally been assumed to be unity (35, 36). However, recent studies with purified recombinant IL-2 and p55 IL-2R, involving cross-
linking experiments, scintillation proximity assays, and affinity chromatography techniques, suggest that this subunit is capable, under certain conditions, of binding two IL-2 molecules.

Structural characterization of both low and high affinity IL-2·IL-2R complexes should contribute to a better understanding of the mechanisms of formation and regulation of the low and high affinity receptor-ligand complexes, as well as of the transduction of this binding event into a physiological response by target cells.

Here, we report the first cocrystallization of a recombinant soluble form of the human p55 component of the high affinity IL-2 receptor with human recombinant IL-2.

**EXPERIMENTAL PROCEDURES**

**IL-2 and IL-2R**— Human recombinant proteins were obtained from Dr. John Smart, Protein Biochemistry, Hoffmann-La Roche Inc. Human recombinant IL-2, expressed in Escherichia coli and in Chinese hamster ovary cells, was produced by Drs. F. Khan, Bioprocess Development and P. Familietti, Molecular Genetics at Hoffmann-La Roche Inc. The β-Nae and 6-Mst soluble recombinant forms of the human p55 IL-2R were produced by Drs. B. Cullen and P. Familietti, Molecular Genetics, and P. Bailon, Protein Biochemistry, Hoffmann-La Roche Inc. (see Fig. 1).

**Desialylation of IL-2R**—1 unit of neuraminidase (from *V. cholerae*, EC 3.2.1.18, Behring Diagnostics) was added to 10 mg of receptor in 1.0 ml of a buffer containing 50 mM sodium acetate, 154 mM NaCl, and 4 mM CaCl₂ at a pH of 5.5. The digestion was allowed to proceed for 2 h at 37 °C and was complete as ascertained by SDS-PAGE and isoelectric focusing. Separation of the desialylated p55 IL-2R from neuraminidase and free sialic acid was done in one step using affinity chromatography on an IL-2 column (see below).

**Purification of Desialylated IL-2R**—The enzyme reaction solution was diluted to 1 mg/ml in PBS and the pH adjusted to 7.4 with NaOH. 1 ml aliquots of this solution were run through a column containing 1 ml of IL-2 affinity sorbent (37) at a flow rate of 1 ml/min at 4 °C. After washing with 10 volumes of PBS, bound material was eluted with 0.2 M acetic acid containing 0.2 M NaCl. The column effluents were monitored by absorbance measurements at 280 nm.

The column was reused after reequilibration with PBS and was stored at 4 °C in PBS containing 0.1% NaN₃.

**Crystallization of the IL-2·IL-2R Complex**—IL-2 and the p55 IL-2R were mixed in 0.1 M ammonium acetate, pH 6.1, and concentrated to a total protein concentration of 10-15 mg/ml using a Centricon 10 microconcentrator (Amicon). The mixture was diluted to 1 mg/ml in PBS and the pH adjusted to 7.4 with NaOH. 1 ml aliquots of this solution were run through a column containing 1 ml of IL-2 affinity sorbent (37) at a flow rate of 1 ml/min at 4 °C. After washing with 10 volumes of PBS, bound material was eluted with 0.2 M acetic acid containing 0.2 M NaCl. The column effluents were monitored by absorbance measurements at 280 nm.

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**Crystallization of the IL-2·IL-2R Complex**—IL-2 and the p55 IL-2R were mixed in 0.1 M ammonium acetate, pH 6.1, and concentrated to a total protein concentration of 10-15 mg/ml using a Centricon 10 microconcentrator (Amicon). The mixture was then allowed to equilibrate for at least 15 min at room temperature prior to being subjected to crystallization conditions. Alternatively, mixtures of receptor-ligand were kept at 4 °C for up to several days in the same buffer.

Crystallization was performed using conventional hanging drop methods (38) and sitting drop variations (39) of the microvapour diffusion technique. The hanging drop method was initially used for screening a wide variety of conditions. In this case, 2 μl of protein was combined with an equal volume of well solution on a siliconized (Prosi 28, SCM Specialty Chemicals) 22-mm glass coverslip which was then inverted and sealed with petroleum jelly on top of a well (24-well Linbro plastic tissue culture plate) containing 1.0 ml of the trial crystallization buffer. The sitting drop method was used for growing crystals under these optimized conditions. Data frames of 0.25 degree of oscillation were collected, using variable crystal-to-detector distance (10-15 cm), swing angle (0-10°), and accumulation time (5-15 min) according to the size and diffraction quality of crystals. Data frames were transferred to a Sun workstation via an Ethernet network and processed with an adapted version of the Xegen program package of A. Howard (41).

**SDS-PAGE Analysis of Coecrystals**—Several crystals were pooled and transferred to 200 μl of a protein-free 1.7 M ammonium sulfate, 0.1 M imidazole solution, at pH 8.0. The crystals were washed extensively by repeated cycles of addition of fresh protein-free solution, mixing, standing at room temperature for several hours, followed by centrifugation and decantation. This process took several days and the crystals were stable under this treatment. The washed crystals and the initial mother liquor were analyzed by SDS-PAGE under both reducing (10 mM dithiothreitol) and boiling for 5 min) and nonreducing conditions in 12.5% acrylamide gels according to Laemmli (42).

**Amino Acid Analysis**—5 to 10 μg of protein or crystals, washed as described above and dissolved in water were placed in acid-washed ignition tubes (10 × 7 mm) and lyophilized. 200 μl of 6 N HCl were added, and the tubes were evacuated to remove dissolved oxygen. The tubes were sealed under vacuum and placed in a heating block at 110 °C for 24 h. The sealed tubes were opened, and the samples were dried under vacuum. The hydrolyzed samples were reconstituted in 250 μl of 0.2 N sodium citrate dilution buffer, pH 2.2 (Beckman), 50-μl aliquots were analyzed on a Beckman model 6300 high performance analyzer (43).

**RESULTS**

Several soluble recombinant forms of the human p55 IL-2 receptor were available for crystallization studies (Fig. 1). β-Nae contains 224 amino acids and lacks exon 5 and most of exon 7, the intracellular and transmembrane portions of the molecule, as well as the 21 N-terminal amino acids forming the signal sequence. 6-Mst contains 179 amino acids, lacks most of exons 5 and 6, which are highly O-glycosylated (23) and contains point mutations that eliminate the consensus

![Fig. 1. Structural map of the p55 IL-2 receptor forms used in this study. Besides lacking the signal sequence (exon 1) the soluble β-Nae form does not contain exon 8, and most of exon 7, corresponding to the cytoplasmic and transmembrane portions of the molecule. 6-Mst further lacks exon 6 and most of exon 5, corresponding to regions that are highly O-glycosylated, and is constructed with point mutations that eliminate the two consensus sequences for N-glycosylation at residues Asn-49 (exon 2) and Asn-68 (exon 3). The regions of the receptor molecule are labeled A, signal peptide; B, extracellular; C, transmembrane; D, cytoplasmic.](image-url)

J. Smart, personal communication.
sequences for the two N-glycosylation sites at Asn-49 and Asn-68 (44). A reducible dimer form of δ-Nae, purified from the expression system, was also used in crystallization experiments (45). These different forms of p55 IL-2R manifest similar binding properties for IL-2 (44, 45).

Under prolonged storage at 4 °C, degradation of the receptor was observed; δ-Mst was the least stable form, resulting in a multitude of smaller fragments, while the δ-Nae dimer showed the greatest stability, showing almost no sign of degradation after several months of storage in 0.1 M ammonium acetate, 0.1% NaN3, pH 5.5-6.1. Storage of a mixture of receptor and ligand, as opposed to receptor alone, was also observed to considerably slow down the degradation process (data not shown). IL-2 alone is stable under the storage conditions used.

Crystals have been obtained from all mixtures of receptor and ligand tested. In all cases optimal crystallization conditions were found to lie around 1.7 M ammonium sulfate, 0.1 M imidazole, pH 7.0-7.7. However, the size and diffraction quality of the crystals varied with the receptor and ligand mixture used. Under all conditions tested, the most promising crystals were obtained with a mixture of the δ-Nae monomer p55 IL-2R combined with unglycosylated IL-2. Also, it was found that crystals grew best when ligand and receptor were present in a 1:1 to 2:1 molar ratio.

Crystals from mixtures of δ-Nae receptor monomer and unglycosylated IL-2 nucleated within 1 week and reached maximal size within 1 month (Fig. 2A). These can grow to 0.8 × 0.4 × 0.4 mm in dimension, possess trigonal or hexagonal morphology, and show very little birefringence. Precession photographs show that the crystals belong to the trigonal space group P3121, or its enantiomorph, as determined from the p2mm symmetry and the systematic absences along the 1-axis in the h01 zone (Fig. 3A) and the absence of mm symmetry in the h0l zone (Fig. 3B). The assignment of the space group is further confirmed by the breakdown of the p6 symmetry of the zero level (hk0) in the first upper level (hk1) where the three strong reflections within the inner Lorentz ring provide clear indication that the crystals are trigonal (Fig. 3C). The cell dimensions are a = b = 91.3 Å and c = 118.3 Å for these crystals. Data for the native crystals have been collected on the area detector to 4 Å resolution, and the search for heavy atom derivatives has been initiated.

In order to further improve the size and quality of the crystals, experiments were done to test the effects of a modification of the oligosaccharide moieties of the receptor molecule. Preliminary experiments indicated that enzymatic removal of N-linked or O-linked oligosaccharides produced less homogeneous forms of the receptor when performed under conditions that did not affect the conformation of the molecule (i.e. without detergent or boiling of the samples; data not shown). However, desialylation of the receptor produced a more homogeneous form of this molecule; the pH of the desialylated δ-Nae receptor monomer moved to 5.7 from 3.5 for the untreated receptor (data not shown). A one-step purification of the desialylated receptor by affinity chromatography on an IL-2 column also demonstrated that the modified receptor still bound IL-2 although no quantitative analysis of binding kinetics was performed.

A mixture of the desialylated p55 IL-2R with unglycosylated IL-2 has so far produced the best quality crystals of the receptor-ligand complex (Fig. 2C). These crystals reach a size of 0.8 × 0.8 × 0.4 mm in 1.7–1.8 M ammonium sulfate, 0.1 M imidazole, pH 7.5–8.4, and thus show an alkaline pH shift when compared with the optimal conditions found when the sialylated receptor is present. A variation in crystal morphology is observed with this crystal form, and this feature is found to be pH dependent. However, desialylation does not seem to be critically involved in the crystal lattice formation as these crystals also belong to the P3121 (or P3221) space group and possess the same cell dimensions (a = b = 91.3 Å, c = 118.3 Å). A significant improvement in the diffraction
Fig. 3. Precession photographs of cocrystals of IL-2 and the p55 IL-2R. A–C, δ-Nae receptor and unglycosylated IL-2 (6.5° precession). A, the h1 zone shows reflections only at positions where 1 = 3n along the 1-axis (horizontal axis), indicative of an hexagonal or trigonal space group. B, the h01 net shows p2 but not mm symmetry and distinguishes between an hexagonal and a trigonal space group. C, the hk1 net shows a breakdown of p6 symmetry and thus confirms the P321 (or P3221) trigonal space group. D, 7.5° precession photograph of a cocrystal of the desialylated δ-Nae receptor and unglycosylated IL-2. The h1 zone shown here and all other zones (not shown) are visually indistinguishable from those of the cocrystals with the fully sialylated receptor. The cell dimensions for both of these crystal forms are a = b = 91.3 Å, c = 118.3 Å.

quality of these crystals is observed, however, and data sets of the native crystals have been collected to 3.5 Å on the area detector. These crystals are stable in the x-ray beam for several days at room temperature. The search for suitable heavy atom derivatives for this crystal form is in progress.

None of the p55 IL-2R forms nor IL-2 crystallizes by itself under similar conditions used successfully for the receptor-ligand mixtures. Nevertheless, in order to verify that both receptor and ligand were incorporated into the crystal lattice, crystals were thoroughly washed and analyzed by SDS-PAGE. Both receptor (44 kDa) and ligand (14 kDa) are indeed found in the crystals (Fig. 4, lane c). In addition, protein fragments of apparent molecular mass of 35 and 25 kDa appear in reducing gels and probably represent degradation products of the desialylated receptor as they are also found in samples containing only the desialylated receptor (lane b). However, when the same material is run under nonreducing conditions, a new band appears at approximately 90 kDa (lane f) and comigrates with the desialylated δ-Nae receptor dimer (Panel B, lane d). These observations are interpreted as representing the result of a covalent dimerization of the δ-Nae receptor molecule, possibly mediated by the formation of a disulfide bridge between the free Cys-192 of each monomer (23). This 90-kDa band is considerably weaker in the mother liquor surrounding the crystallized complex (lane g, versus lanes f and h).

In order to determine further the contents of the crystals, an analysis of the amino acid composition of the crystallized material was performed to provide an indication of the IL-2 to IL-2 receptor stoichiometry. Table I shows compositions determined for IL-2 and the δ-Nae p55 IL-2R as well as compositions expected and calculated for the presence of receptor and ligand in a 1:1 and 1:2 ratio. Overall, the amino acid composition of the crystals shows a slightly better agree-

Fig. 4. Panel A, SDS-PAGE analysis, under reducing conditions, of the desialylated δ-Nae IL-2 receptor dimer (lane a), the desialylated δ-Nae IL-2 receptor monomer (lane b), and of crystals of the complex between the receptor and unglycosylated IL-2 (lane c). Prior to being run on the gel, the crystals were washed extensively and allowed to exchange interlattice solvent with protein-free mother liquor for several days. Both forms of the desialylated receptor (44 kDa) show major degradation products of apparent molecular mass of 35 and 25 kDa. Lane c shows the presence of the receptor, its smaller fragments and IL-2 (14 kDa). Thus, both receptor and ligand are present in these crystals. Panel B, SDS-PAGE analysis, under nonreducing conditions, of the desialylated δ-Nae receptor dimer (lane d), the δ-Nae receptor monomer (lane e), and of the contents of the cocrystals of the desialylated δ-Nae receptor monomer and unglycosylated IL-2 (lane f). Panel C, SDS-PAGE analysis, under nonreducing conditions, of the initial receptor-ligand mixture put into a crystallization well (lane g) and of washed crystals (lane h).
that it is not possible to fit an amino acid composition for the crystals that agrees with values expected for leucine and any other amino acid if a 1:2 ratio is assumed.

**DISCUSSION**

Cocrystals of various forms of both recombinant human IL-2 and a soluble recombinant form of the human p55 component (Tac) of the IL-2 receptor have been obtained from protein mixtures in ammonium sulfate. We have initiated x-ray diffraction data collection and analysis with crystals of the complex between the desialylated receptor and unglycosylated IL-2, which diffract to around 3.5 Å. Concomitantly, we are pursuing the refinement of crystallization conditions for other combinations of the various forms of the receptor and ligand, as well as for the receptor and IL-2 by themselves.

The search for suitable heavy atom derivatives is in progress, including those that have proven successful in the recently reported structure determination of the IL-2 molecule (10).

The improvement observed in the diffraction quality of the crystals involving the desialylated receptor may be due to the increased homogeneity in weight and charge of the molecule following the enzymatic treatment. It may also reflect reduced constraints that the bulky and charged terminal sialic acid residues could impose on the formation of the crystal lattice. Multiple experiments with the variously glycosylated forms of the receptor and ligand molecules have indicated that the diffraction quality of the crystals inversely correlates with the overall degree of glycosylation. The δ-Mst receptor molecule, which contains less of the O-linked and no N-linked oligosaccharide moieties, suffers, however, from a fast rate of degradation and has not yet proved superior in the formation of cocrystals. We have nevertheless obtained microcrystals of the δ-Mst molecule by itself and are currently improving these crystallization conditions.

Analysis of the protein content of the crystals by SDS-PAGE provides strong evidence that receptor and ligand coexist in the crystal lattice. Yet, we cannot from these studies strictly distinguish between the occurrence of a simple co-crystallization versus the crystallization of a biologically relevant complex between receptor and ligand. However, the phenomenon of crystallization itself requires that molecules be rather tightly packed and highly ordered; it thus seems improbable that the receptor and its specific ligand would cocrystallize in an arrangement that does not take advantage of their complementary surface and mutual affinity. Published structures of other types of macromolecular complexes support this assumption (46-48).

Surprisingly, our results show evidence for a covalent reducible dimer of the p55 IL-2 receptor in the cocrystals of the desialylated receptor monomer and IL-2. This band was almost completely absent from the mother liquor in the crystallization droplets and has never been observed in our frequent analyses of receptor-ligand mixtures nor solutions of receptor monomer alone. This significant enrichment in the dimer form may be the result of a preferential incorporation of the receptor dimer which could form transiently in the protein solution under the crystallization conditions. Alternatively, the dimerization reaction may occur within the crystal lattice, possibly as a result of a particularly favorable positioning of the free Cys residues thought to be involved in the formation of the disulfide linkage. Cocrystallization experiments using IL-2 and a purified reducible dimer of the IL-2 receptor, whether it is desialylated or not, have yielded microcrystals (0.1-mm long hexagonal rods). These observations do not, however, distinguish between the possible routes for dimerization of the receptor molecule.

The amino acid content of the crystals suggests that, overall, the δ-Nae form of the p55 IL-2 receptor and IL-2 exist in a 1:1 ratio. However, although these results are consistent with previously reported cross-linking studies which indicate that IL-2 and the p55 IL-2R bind in a 1:1 ratio (29-36), the possibility that a particular packing of the crystals involves both receptor-ligand complex and free receptor cannot be ruled out at this time. In addition, the presence of several fragments of the receptor in the crystal lattice also prevents a definitive correlation between the amino acid composition in the crystals and the true stoichiometry of the binding of IL-2 to the p55 IL-2 receptor. The calculated value for the

### Table I

Amino acid composition of cocrystals of δ-Nae IL-2R and IL-2

| Amino acid | IL-2, found (predicted) | δ-Nae IL-2R, found (predicted) | IL-2/IL-2R | IL-2/IL-2R | Difference | Difference | Founda |
|------------|------------------------|--------------------------------|-------------|-------------|------------|------------|--------|
| Asx        | 12.4 (12)              | 11.8 (11)                      | 24.2        | 12.2        | +2.0       | +0.3       | 36.5   |
| Thr        | 11.0 (13)              | 21.2 (25)                      | 32.2        | 30.5        | +1.7       | -0.6       | 42.4   |
| Ser        | 5.8 (6)                | 15.8 (16)                      | 22.7        | -1.0        | 21.4       | 29.0       | 44.4   |
| Glx        | 10.8 (15)              | 43.3 (41)                      | 62.1        | -1.7        | 60.4       | 38.8       | 84.1   |
| Pro        | 4.9 (5)                | 18.3 (18)                      | 23.2        | -2.9        | 20.3       | 28.1       | 30.3   |
| Gly        | 2.3 (2)                | 12.6 (12)                      | 14.9        | +0.9        | 15.8       | 17.2       | 22.0   |
| Ala        | 5.4 (5)                | 12.3 (13)                      | 18.3        | -0.1        | 18.2       | 23.7       | 25.3   |
| Val        | 3.6 (4)                | 9.2 (10)                       | 12.9        | -0.3        | 12.6       | 16.5       | 17.5   |
| Met        | 4.1 (4)                | 8.0 (10)                       | 12.1        | -1.3        | 10.8       | 16.2       | 15.0   |
| Ile        | 7.0 (9)                | 4.9 (6)                        | 11.9        | +0.3        | 12.2       | 18.9       | 17.0   |
| Leu        | 23.2 (22)              | 8.9 (8)                        | 32.1        | +1.5        | 33.6       | 55.3       | 46.8   |
| Phe        | 5.8 (6)                | 5.5 (6)                        | 11.3        | -0.2        | 11.1       | 17.1       | 15.4   |
| Lys        | 15.9 (11)              | 12.5 (10)                      | 26.2        | +4.6        | 30.8       | 40.1       | 42.9   |
| Total      | 119.0 (119)            | 184.8 (186)                    | 303.8       | 303.9       | 422.8      | 423.0      |        |

a Cys, Trp, His, and Arg were not determined in this system. Data presented are averaged over three amino acid hydrolyses.

b No correction was made to compensate for over- or underhydrolysis.

c Calculated from averaged composition of IL-2 and δ-Nae IL-2R. This provides an adequate correction for the analysis of mixtures of receptor and ligand.

d Calculated with the assumption that the total amino acid composition corresponds to that of an integral 1:1 or 1:2 receptor to ligand ratio.
Matthews coefficient with one 1:1 receptor-ligand complex/ asymmetry unit is 2.7, yielding a solvent content of 45%, typical of protein crystals (49).

Our analysis of the cocrystals indicates the presence of a heterogeneous population of the receptor molecule. The SDS-receptor-ligand complex which may involve several coexisting complex structures can then be used to better define strategies for the design of agonist and antagonist drugs of potentially valuable medical importance.

The crystallization, x-ray diffraction, and crystal analysis data presented here indicate promise for identifying the important processes involved in the immunology and cell biology of IL-2.

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