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# Identification of specific residues of human interleukin 2 that affect binding to the 70-kDa subunit (p70) of the interleukin 2 receptor

**(site-directed mutagenesis/competitive binding/circular dichroism)**

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**ABSTRACT** **Analogs of interleukin 2 containing defined amino acid substitutions and deletions were assayed for bioactivity and for competitive binding to the high-affinity human interleukin 2 receptor complex and its two component subunits, a 55-kDa subunit (p55 or TAC) and a 70-kDa subunit (p70). Substitution of $Asp^{20}$ or deletion of $Phe^{124}$ resulted in inactive analog proteins that were unable to interact with the high-affinity p55/p70 complex or the intermediate-affinity p70 subunit of the interleukin 2 receptor. These analogs, however, retained the capacity to compete for binding to the low-affinity p55 subunit. The presence of the carboxylic acid in the side chain of $Asp^{20}$ was necessary for effective binding to the p70 protein. In contrast, substitution of $Trp^{121}$ and $Leu^{17}$ created analogs that were inactive in the bioassay and all three binding assays. The effects of these mutations on protein conformation were assessed by circular dichroism. These results demonstrate that specific residues in the $NH_2$ and COOH termini of interleukin 2 are crucial for its structure and activity.**

Human interleukin 2 (huIL-2) exerts immunoregulatory effects on a variety of cells including T cells, activated B cells, natural killer cells, and lymphokine-activated killer cells. The biological effects of huIL-2 are mediated through specific interactions with cell surface receptors present on the target cells (1, 2). High-, intermediate-, and low-affinity forms of the IL-2 receptor (IL-2R) have been identified on these cells. The high-affinity receptor exhibits an apparent $K_d$ of $\approx 10^{-11}$ M and exists as a complex of at least two distinct polypeptides, with molecular masses of 55 kDa (p55 or Tac) and 70–75 kDa (p70 or p75). Cells or cell lines expressing only p55 are capable of binding IL-2 (3, 4). These cells have been used to demonstrate that the p55 subunit corresponds to the low-affinity form of the receptor ($K_d = 1–3 \times 10^{-8}$ M). It has been reported that the p70 protein, which is expressed at relatively high levels on the human YT-1 cell line (5), can also bind IL-2 and corresponds to an intermediate-affinity ($K_d = 8.2 \times 10^{-10}$ M) receptor. Thus, unlike many multisubunit receptors, each of the two subunits of the IL-2R can be expressed at the cell surface and can bind ligand in the absence of the other chain.

The biological consequences of these binding interactions have been studied on various target cells. The p70 protein (6–8) is present on resting T cells, natural killer cells, and lymphokine-activated killer cell precursors. Binding of IL-2 to p70 allows internalization of IL-2, induces *de novo* synthesis and expression of p55 molecules, and triggers the development of increased cytolytic activity in natural killer cells and an early phase of effector lymphokine-activated killer cells (6, 7, 9, 10). The induction of p55 allows assembly of the p55/p70 complex (i.e., the high-affinity IL-2R) that appears to be essential for a full proliferative response by T cells and for the late phase of effector lymphokine-activated killer functions.

To understand the structure–function relationship of huIL-2 and the interactions with its receptor, we have endeavored to identify those amino acids that mediate binding to the high-affinity receptor as well as to its two component subunits. By using site-directed mutagenesis, we had engineered (11) a series of huIL-2 analogs by the introduction of specific deletions and substitutions to modify the protein. The mutant huIL-2 proteins were produced in *Escherichia coli* and assayed for biological activity. We have now extended these studies with additional analogs and assayed their ability to compete for binding to the IL-2R and its subunits. The identification of specific amino acids required for bioactivity and binding to the p55 and p70 subunits has allowed us to correlate the structure of IL-2 with its biological functions.

## METHODS AND MATERIALS

**Bacterial Strains and Plasmids.** All plasmids were propagated in *E. coli* strain MC1061 transformed with the low-copy-number compatible plasmid pRK248*cI*ts, which carries the gene for the temperature-sensitive repressor of the phage $\lambda$ $P_L$ promoter (12). For preparation of undermethylated DNA for restriction analysis, plasmids were grown in GM119, a *dam*$^-$ mutant of *E. coli*.

**Enzymes.** All restriction endonucleases, *E. coli* polymerase (Klenow fragment), T4 DNA ligase, and T4 polynucleotide kinase were obtained from New England Biolabs.

**Preparation and Analysis of IL-2 Analog Proteins.** The procedure utilized for introduction of site-directed mutations was as described (11). Extracts of *E. coli* cells expressing IL-2 analogs were prepared as described (11) except the final concentration of $NaDodSO_4$ in the Laemmli sample buffer was 0.5%. Analog proteins were analyzed on 15% polyacrylamide gels. The bioactivity of each sample was determined with the IL-2-dependent murine T-cell line CTLL-2. All bioassays were quantitated by using the National Institutes of Health, Bureau of Biological Response Modifiers Reference Reagent Human IL-2 as a standard.

Competitive binding to the high-affinity IL-2R was performed as described (11) except activated human peripheral blood lymphocytes were used. The intermediate-affinity p70 receptor assay was performed with YT-1 cells in the presence of anti-Tac antibody by the method essentially as described by Robb *et al.* (5). The low-affinity soluble receptor binding assay was performed according to the method of Hakimi *et al.* (13). For these assays, samples were solubilized in

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Abbreviations: hu, human; IL-2, interleukin 2; IL-2R, interleukin 2 receptor.

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Table 1. Bioactivity of huIL-2 analogs

| Region | Mutation | % bioactivity |
|---|---|---|
| $NH_2$ terminus | $Asp^{20} \rightarrow Lys$ | <0.1 |
| | $Asp^{20} \rightarrow Asn$ | 37.5 |
| Internal | $Asn^{30} \rightarrow Glu$ | 50–75 |
| | $Lys^{32} \rightarrow Glu$ | 50–100 |
| | $Asn^{33} \rightarrow Asp$ | 100 |
| | $Pro^{34} \rightarrow Gly$ | 19–37 |
| | $Thr^{37} \rightarrow Ile$ | 67 |
| | $Met^{39} \rightarrow Gln$ | 100 |
| | $Phe^{42}$ and $Phe^{44} \rightarrow Tyr$ and Tyr | 100 |
| | $Phe^{42}$ and $Phe^{44} \rightarrow Ala$ and Ala | 0.4–1.2 |
| | $Pro^{47} \rightarrow Gly$ | 19–25 |
| | $Thr^{51} \rightarrow Ile$ | 75–100 |
| | $Glu^{52} \rightarrow Lys$ | 100 |
| | $Leu^{53} \rightarrow Asn$ | 50–100 |
| | $Leu^{56} \rightarrow Met$ | 12 |
| | $Leu^{56} \rightarrow Gly$ | 1–12 |
| | $Leu^{56} \rightarrow Asn$ | 0.4–1.2 |
| | $Gln^{57} \rightarrow Glu$ | 4–40 |
| COOH terminus | Delete $Phe^{124}$–$Gln^{126}$ | <0.1 |
| | Delete $Phe^{124}$ | <0.13 |
| | Delete $Ser^{127}$–$Ile^{129}$ | 3–13 |

Bioactivity was calculated as percent activity as compared to an extract of wild-type huIL-2, measured on murine CTLL-2 cells. All mutants expressed amounts of IL-2 analog protein comparable to the wild-type protein (11).

Laemmli buffer at a final $NaDodSO_4$ concentration of 0.5% and assayed in 2-fold serial dilutions.

**Purification of IL-2 Analog Proteins.** The $Lys^{20}$ analog was purified by receptor affinity chromatography with immobilized p55 protein. All other analogs were purified by immunoaffinity chromatography with a murine monoclonal antibody (5B1) that binds recombinant huIL-2. Both of these methods have been described by Bailon *et al.* (14).

**Circular Dichroism (CD) Analysis.** CD spectra were determined at room temperature on a Jasco (Easton, MD) J-20 automatic recording spectropolarimeter. Spectral bandwidth was set at 1 nm. Cuvettes of 0.01 cm and 1 cm in light path length were used for 190–260 nm and 250–350 nm, respectively. All samples were analyzed in buffer containing 50 mM NaOAc, pH 3.5, and mannitol (5 mg/ml), with the buffer spectrum subtracted from the protein spectrum. Results were calculated assuming a molecular mass of 15 kDa for huIL-2 and its analogs.

## RESULTS

By using synthetic oligonucleotides, a series of recombinant huIL-2 analogs was produced by site-directed mutagenesis of the huIL-2 *E. coli* expression plasmid pRC233/IL-2/Δtet (11). We have now characterized an additional 21 analogs (Table 1), resulting in the analysis of a total of 81 mutations of the huIL-2 gene. Representative clones of each of the analogs were assayed for residual bioactivity on the murine T-cell line CTLL-2 as described (11) and compared to wild-type huIL-2 prepared in a similar manner. As noted in our previous study (11), the majority of these analogs retained full biological activity, suggesting that a substantial portion of the IL-2 molecule can tolerate amino acid substitutions without affecting its activity.

Several analogs, however, had significantly diminished bioactivity and were, therefore, analyzed further. In the $NH_2$ terminus, replacement of $Asp^{20}$ with Lys resulted in a substantial loss of bioactivity, to <0.1% residual activity. At the COOH terminus, a marked reduction in bioactivity was also observed when we analyzed analog proteins containing a small deletion of residues 124–126 (Phe-Cys-Gln). Deletion of only $Phe^{124}$ caused a similar decrease of bioactivity (<0.13%). A deletion of residues 127–129 had a less dramatic effect, causing a reduction of bioactivity to 3–13%. These mutations highlight the importance of the $NH_2$ and COOH termini for IL-2 bioactivity.

Some monoclonal antibodies that neutralized huIL-2 bioactivity recognized epitopes that mapped to an internal region between residues 30 and 60 (15). However, in our studies, extensive mutagenesis of this region, including substitution of each of the residues between positions 29 and 55, did not identify any additional amino acids that were crucial for maintaining bioactivity except replacement of $Phe^{42}$ and $Phe^{44}$ with alanines (Table 1). A significant decrease in activity was also observed when $Leu^{56}$ was replaced with Met, Gly, or Asn, or when $Gln^{57}$ was changed to Glu. These two amino acids are located adjacent to the $Cys^{58}$ residue, which forms an essential disulfide bond with $Cys^{105}$ (16). Therefore, it is possible that substitutions at these positions had an indirect effect on bioactivity by preventing correct disulfide formation, thus altering the folding or

Table 2. Bioactivity versus competitive binding of huIL-2 analogs

| Analog | Bioactivity, units/mg | Bioactivity, % of wild type | % competitive binding, p55/p70 | % competitive binding, p55 | % competitive binding, p70 |
|---|---|---|---|---|---|
| Purified | | | | | |
| Wild type | $2 \times 10^7$ | 100 | 100 | 100 | 100 |
| $Asp^{20} \rightarrow Lys$ | $2.7 \times 10^4$ | 0.135 | 0.115, 0.30 | 100 | 0.14, 0.2 |
| $Trp^{121} \rightarrow Ser$ | $3 \times 10^4$ | 0.15 | 0.08 | <0.26 | 0.39 |
| $Cys^{58} \rightarrow Ser$ | $2.7 \times 10^4$ | 0.135 | 0.1 | <0.26 | 1.03, 1.3 |
| $Leu^{17} \rightarrow Asn$ | $3 \times 10^5$ | 1.6 | 0.39 | 1 | 1.2, 1.54 |
| Crude | | | | | |
| $Asp^{20} \rightarrow Asn$ | ND | 37.5 | 0.316 | 100 | <0.1 |
| Delete $Phe^{124}$–$Gln^{126}$ | ND | <0.002 | <0.001 | 19–34.5 | <10 |
| Delete $Phe^{124}$ | ND | <0.13 | <0.1 | 27 | 0.15 |
| Delete $Ser^{127}$–$Ile^{129}$ | ND | 19–37.5 | 5–24 | 25–50 | ND |
| Delete $Ala^1$–$Asp^{20}$ | ND | <1 | <1 | <10 | ND |
| $Leu^{17} \rightarrow Val$ | ND | 5 | 2 | <10 | ND |
| $Leu^{56} \rightarrow Met$ | ND | 37.5 | 20 | 30–37 | 26, 28 |
| $Gln^{57} \rightarrow Glu$ | ND | 33–50 | 38–42 | 12.5–16 | ND |

Percent bioactivity and competitive binding were calculated from $IC_{50}$ values as described (11) and in the legend to Fig. 1. The bioactivity was measured on murine CTLL-2 cells. The p55/p70 % competitive binding was measured on activated human peripheral blood lymphocytes. The p55 % competitive binding was measured on purified immobilized human p55. The p70 % competitive binding was measured on human YT-1 cells in the presence of anti-Tac. ND, not determined.

stability of the huIL-2 protein, rather than altering a specific residue that binds directly to the IL-2R.

To distinguish the effects of specific mutations on the conformational aspects of IL-2 structure versus mutations that directly affected receptor interactions, selected analogs were tested in three competitive binding assays. Each analog was examined for its capacity to compete with radiolabeled wild-type huIL-2 for binding to the high-affinity (p55/p70) human IL-2R, the low-affinity (p55) receptor subunit, or the intermediate-affinity (p70) subunit. The competitive binding assays for the high- and intermediate-affinity receptors were performed with whole cells (5, 11), whereas competitive binding to the low-affinity receptor was performed with purified soluble p55 protein (13). For each analog, the percent competitive binding relative to wild-type huIL-2 was calculated as described (11) and compared to the percent bioactivity (Table 2). For selected experiments, four analogs were purified to apparent homogeneity; we have observed no differences in the percent bioactivity or competitive binding activity of purified material compared with crude extracts when equivalent amounts of protein were assayed.

It is important to note that bioactivity was measured on the standard mouse CTLL cell line, whereas competitive binding was measured with human IL-2R and its subunits. The murine cell line was chosen for the following two reasons: (*i*) IL-2 bioactivity, in units as defined by the National Institutes of Health standard, is assayed by using this target cell; and (*ii*) no human cell line has been identified that is biologically equivalent to the CTLL line (i.e., absolutely dependent upon exogenous IL-2 for proliferation).

In almost all cases tested, the present residual bioactivity of each analog correlated directly with the percent competitive binding to the human p55/p70 high-affinity receptor complex (Table 2). However, the comparison of bioactivity with competitive binding to either p55 or p70 revealed interesting differences with several analogs. Crude extracts of the $Lys^{20}$ analog were originally observed to have very low residual bioactivity (<0.1%, Table 1). This analog, when purified to homogeneity on a p55 receptor affinity column (14), exhibited the same low level of bioactivity (0.135%) and correspondingly low percent binding to p55/p70 (0.115–0.3%) and to p70 (0.14–0.2%) (Table 2 and Fig. 1 *A* and *C*). In contrast, this analog was able to compete for p55 binding with an $IC_{50}$ and a binding curve indistinguishable from wild-type huIL-2 (Fig. 1*B*). These findings suggest that $Asp^{20}$ may participate directly in forming a part of the binding site for p70. Substitution of asparagine at position 20 caused a partial loss of bioactivity (37.5%), but, as expected, this analog was equivalent to wild-type huIL-2 in its ability to compete for binding to p55 (Table 2).

To determine the effects of the $Lys^{20}$ substitution on overall protein conformation, CD analysis was performed. The $Lys^{20}$ analog produced a CD profile identical to that of the wild-type protein (Fig. 2*A*), showing that its conformation had not been grossly altered. The CD spectra of wild-type and $Lys^{20}$ proteins indicated that both samples had a high degree of $\alpha$-helical content ($\approx$70%), in agreement with a previous report describing CD results obtained with a refolded recombinant huIL-2 analog (17).

A second analog deleted at residues 124–126 near the COOH terminus was observed to have a similar profile of bioactivity and binding activities as the $Lys^{20}$ analog. This analog was inert in the bioassay (<0.002%) (Table 2) and was also inactive in competitive binding assays for the high-affinity (<0.001%) and p70 (<10%) receptors. However, this analog showed significant activity in the competitive binding assay for p55 (19–34.5%). Deletion of only $Phe^{124}$ also created an analog with very similar biochemical and biological properties. Therefore, in addition to $Asp^{20}$, the region around residues 124–126 may also play a role in interaction with the p70 subunit. Purification of the $Phe^{124}$ deletion analog is in progress to evaluate the effects of this mutation on protein conformation.

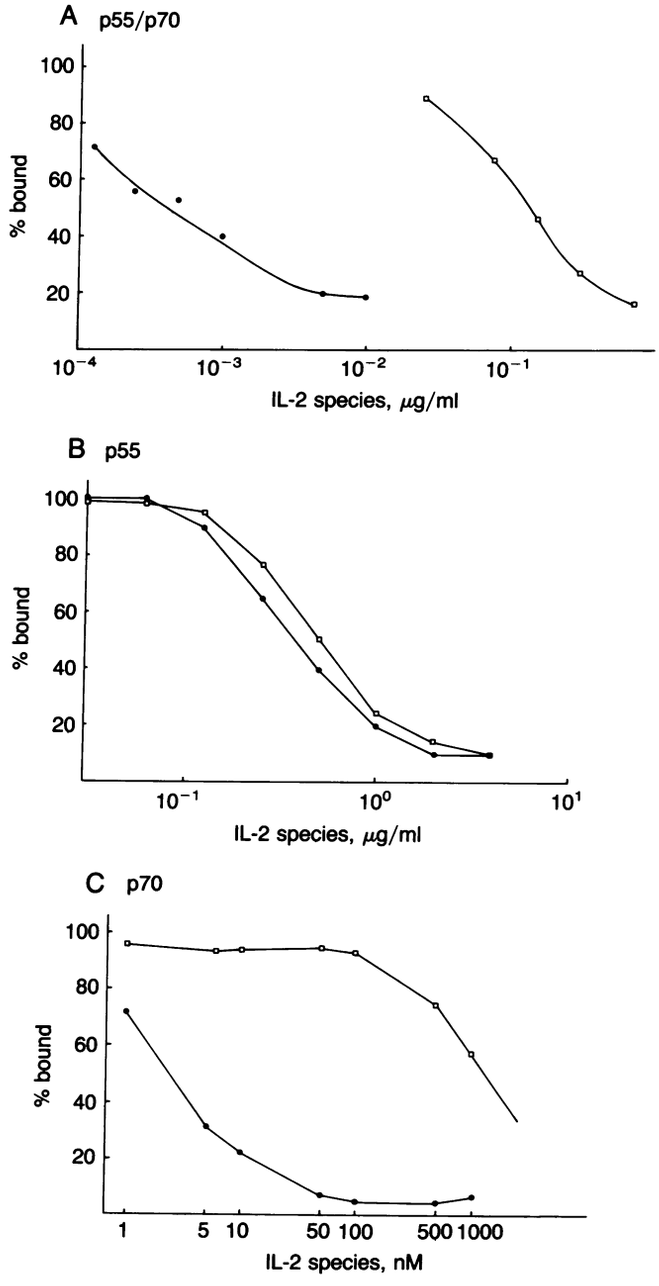


FIG. 1. Competitive binding of the $Lys^{20}$ analog to the IL-2R and its subunits. Intact human peripheral blood lymphocytes (*A*) or human YT-1 cells (*C*) were incubated with 40 pM or 1 nM $^{125}$I-labeled huIL-2, respectively, in the presence of various concentrations of purified wild-type huIL-2 protein (●) or the $Lys^{20}$ analog (□). In the solid-phase assay (*B*), immobilized purified human p55 protein was incubated with biotinylated huIL-2 (100 ng/ml) in the presence of various concentrations of wild-type or $Lys^{20}$ proteins. Data represent binding as a percentage of that observed in the absence of any competing ligand. In each assay, the $IC_{50}$ (concentration of protein required to inhibit binding to radiolabeled huIL-2 by 50%) was determined and used to calculate the percent competitive binding (see ref. 11) of the $Lys^{20}$ analog as compared to the wild-type huIL-2 protein. The percent values are shown in Table 2. Competitive binding to the high-affinity p55/p70 (*A*), low-affinity p55 (*B*), and intermediate-affinity p70 (*C*) components of the human IL-2R is shown.

An examination of eight other analogs did not identify any

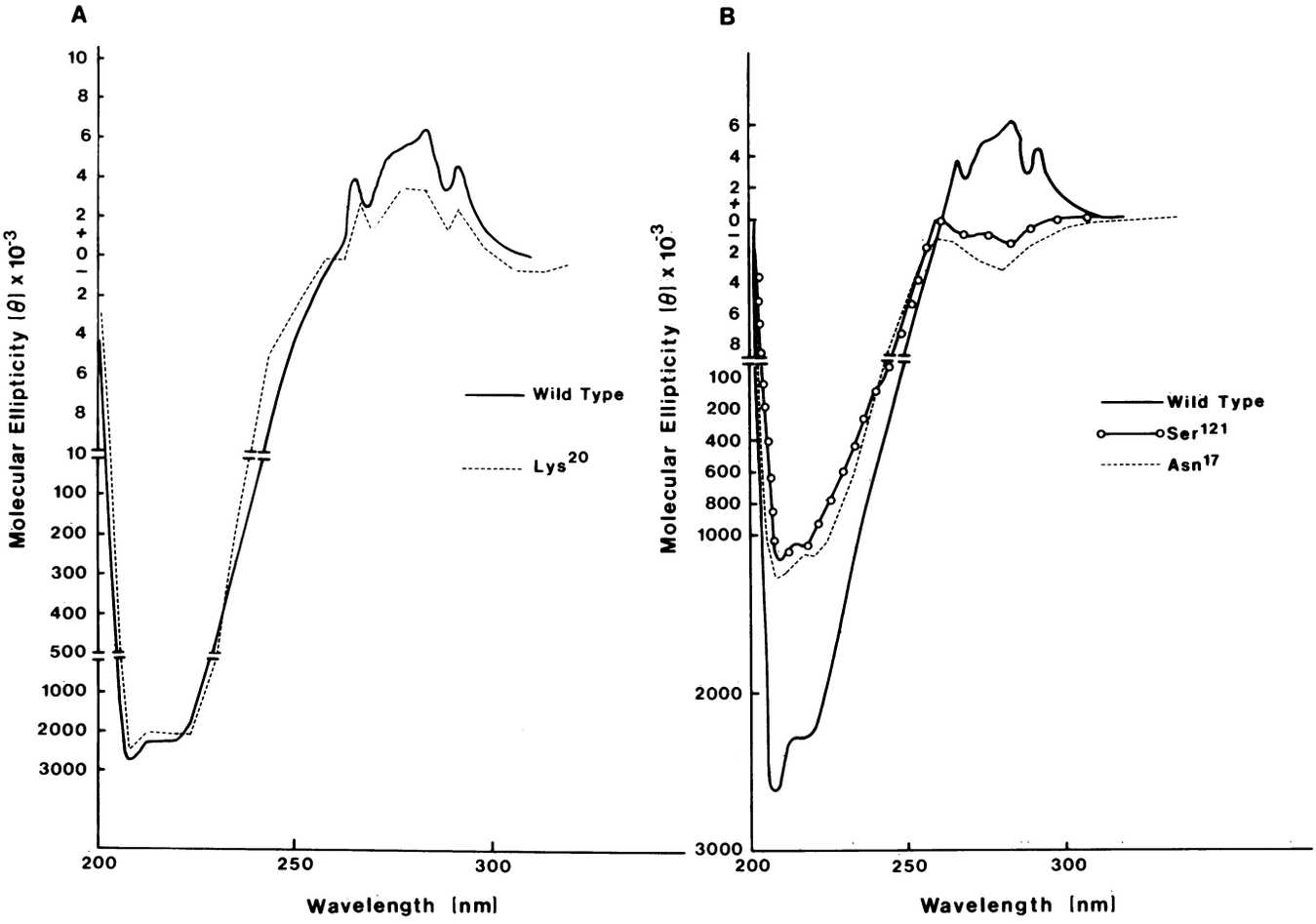


FIG. 2. CD spectra of huIL-2 analogs. All proteins were in a buffer of 50 mM NaOAc, pH 3.5, and mannitol (5 mg/ml). Note differences in scale of ordinates for molecular ellipticities. (*A*) Solid line, wild-type huIL-2; dashed line, $Lys^{20}$ analog. (*B*) Solid line, wild-type huIL-2; o, $Ser^{121}$ analog; and dashed line, $Asn^{17}$ analog.

additional residues or regions of huIL-2 involved in receptor binding (Table 2). In particular, analogs such as those containing $Ser^{121}$ and $Asn^{17}$ substitutions had low bioactivities that correlated with low activities in all three receptor binding assays. To assess the effects of these substitutions on protein conformation, these analogs were purified and analyzed by CD (Fig. 2*B*). Both $Ser^{121}$ and $Asn^{17}$ analogs exhibited CD spectra different from that of wild-type huIL-2. The $\alpha$-helical content of each analog was reduced at least 50% compared to the wild-type protein (compared ellipticities between 200 nm and 250 nm), suggesting that the conformation of these proteins were severely altered. These altered CD spectra are similar to the spectra obtained with huIL-2, which was partially unfolded in 8 M urea (17).

## DISCUSSION

By using site-directed mutagenesis, a series of analogs of huIL-2 has been created that contain defined amino acid substitutions and deletions. We had reported (11) that residues $Leu^{17}$, $Trp^{121}$, $Cys^{58}$, and $Cys^{105}$ were essential for IL-2 bioactivity. In this report, we have identified additional residues that are required for activity, specifically $Asp^{20}$ and the COOH-terminal region involving residues 124–126. Mutations of these amino acids resulted in analog proteins that had <0.2% residual bioactivity compared to wild-type huIL-2.

We have extended these studies to analyze the effect of these mutations on receptor interactions. The substitution of lysine at position 20 created an analog protein that had lost competitive binding to the p55/p70 complex as well as the p70 subunit but had retained substantial binding to the p55 subunit. Scatchard analysis with $^{125}$I-labeled $Lys^{20}$ protein indicated that this analog did not bind to human p55/p70 or p70, but did have a $K_d$ of 3 nM for the soluble p55 receptor and a $K_d$ of 9 nM for the low-affinity receptor on YT-1 cells (data not shown). Deletion of $Phe^{124}$–$Gln^{126}$ or $Phe^{124}$ only resulted in proteins with properties similar to the $Lys^{20}$ analog. These results indicate that $Asp^{20}$ and residues in the $Phe^{124}$–$Gln^{126}$ region participate in the formation of the binding site for p70 on the IL-2 molecule. Additional conservative substitutions of glutamic acid (11) or asparagine at position 20 (which resulted in 100% and 37.5% residual bioactivity, respectively) confirm that a carboxylic acid group in the side chain of this residue is essential for full bioactivity, possibly for a direct ionic interaction with a specific residue on the p70 protein.

Alternatively, either the $Asp^{20}$ substitution or the $Phe^{124}$ deletion might alter the tertiary conformation or the stability of the protein, thereby eliminating bioactivity and binding. We used CD to measure the effects of the $Lys^{20}$ substitution on IL-2 conformation. The CD spectra obtained (Fig. 2*A*) for wild-type huIL-2 and the $Lys^{20}$ analog were identical and very similar to the spectra reported for another analog of IL-2 (17). These results supported our conclusion that $Asp^{20}$ is directly involved in p70 interaction, rather than in maintaining the overall structure of huIL-2.

In contrast to the $Asp^{20}$ substitution, the deletion of

residues 124–126 is more likely to produce a major structural alteration. We have proposed (11) that the COOH terminus may play a structural role in the conformation of IL-2 rather than a direct role in binding. Deletion of $Phe^{124}$, $Cys^{125}$, or $Gln^{126}$ has been reported to eliminate bioactivity (18, 19); however, no data have been presented concerning their effects on binding to the human IL-2R or its subunits. Because deletion of only $Phe^{124}$ caused a dramatic reduction in activity, whereas substitution of $Cys^{125}$ or $Gln^{126}$ did not affect bioactivity (11), it seems likely that $Phe^{124}$ is the critical residue required for full bioactivity and binding. Our finding that the $Phe^{124}$ deletion impaired high-affinity and p70 receptor binding but had less marked effects on p55 binding raises the possibility that the COOH-terminal region is required for maintaining the p70 binding site but not for the p55 binding site.

The importance of the $NH_2$ terminus in p70 interaction has been suggested by epitope mapping of neutralizing monoclonal and polyclonal antibodies. Antibodies that recognize residues 8–26 can neutralize huIL-2 bioactivity (15) and can also block huIL-2 from binding to the p70 subunit of the IL-2R (5). Our studies specifically identify $Asp^{20}$ as one of the critical amino acids in the $NH_2$ terminus required for p70 binding.

In contrast with the results obtained with the $Asp^{20}$ and $Phe^{124}$ mutations, other analogs that showed decreased bioactivity had also lost the ability to compete for binding to the high-affinity IL-2R and both the p55 and p70 subunits (Table 2). These data are consistent with the conclusion that substitutions at $Leu^{17}$, $Trp^{121}$, and $Cys^{58}$ resulted in the production of analogs with altered conformation or reduced stability. The altered conformation of two of these analogs was confirmed by CD analysis. Thus, a comparison of bioactivity with the capacity to bind to each subunit clearly allows us to distinguish those residues involved directly in receptor interactions from residues that play a role in maintaining the overall structure of the IL-2 protein.

Mutational analyses have not yet resulted in the identification of those residues of huIL-2 that interact with the p55 subunit. Our analysis of analogs created by substitution of each of the residues from positions 29 to 55 (ref. 11 and this report) did not indicate any mutations resulting in a substantial reduction in bioactivity except replacement of $Phe^{42}$ and $Phe^{44}$ with alanines (see below). Substitutions for $Leu^{56}$ or $Gln^{57}$ did cause a partial loss in residual bioactivity, but competitive binding to p55/p70, p55, or p70 was reduced to the same degree. Mutagenesis of more than one residue per molecule may be necessary to disrupt binding to p55.

The conclusions we have drawn from site-directed mutagenesis can now be related to the three-dimensional structure of huIL-2 as determined by x-ray crystallography (20). Our identification of $Asp^{20}$ as the contact residue for p70 orients the first $\alpha$-helical bundle, called the A helix, as interacting with this subunit of the IL-2R. $Leu^{17}$ is also located in the A helix and may be crucial for maintaining the conformation of this helix. $Trp^{121}$ and $Phe^{124}$ are located in the F helix, which is proposed to act as part of a structural scaffold. The side chains of both these residues are on the internal face of this helix and are in close proximity to $Phe^{42}$ in the C helix; all three side chains appear to interact with each other (D. McKay, personal communication). This scaffolding model is supported by the observed loss of bioactivity when $Phe^{42}$ and $Phe^{44}$ are replaced with alanines. At this time, our data do not implicate any of the residues of the B′ helix as important for p55 binding. The hinge between the B and B′ helix appears to be flexible because the $Pro^{47} \rightarrow$ Gly analog retained significant activity (19–25%).

Further mutagenesis of huIL-2 will be necessary to determine the validity of this model and to strengthen our conclusions on the function of the relevant residues on bioactivity and receptor interactions. An additional result of this work is the availability of analogs of IL-2 that preferentially interact with the p55 protein. Because these analogs allow us to determine the consequences of p55 binding versus p70 binding on lymphoid cells, we can assign biological functions directly to the IL-2R and its individual subunits.

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1. Robb, R. J., Munck, A. & Smith, K. A. (1981) *J. Exp. Med.* **154,** 1455–1474.
2. Robb, R. J., Greene, W. C. & Rusk, C. M. (1984) *J. Exp. Med.* **160,** 1126–1146.
3. Greene, W. C., Robb, R. J., Svetlik, P. B., Rusk, C. M., Depper, J. M. & Leonard, W. J. (1985) *J. Exp. Med.* **162,** 363–368.
4. Hatakeyama, M., Minamoto, S., Uchiyama, T., Hardy, R. R., Yamada, G. & Taniguichi, T. (1985) *Nature (London)* **318,** 467–470.
5. Robb, R. J., Rusk, C. M., Yodoi, J. & Greene, W. C. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 2002–2006.
6. Seigel, J. P., Sharon, M., Smith, P. L. & Leonard, W. J. (1987) *Science* **238,** 75–78.
7. Tsudo, M., Goldman, C. K., Bongiovanni, K. F., Chan, W. C., Winton, E. F., Yagita, M., Grimm, E. A. & Waldmann, T. A. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5394–5398.
8. Dukovich, M., Wano, Y., Thuy, L. t. B., Katz, P., Cullen, B. R., Kehrl, J. H. & Greene, W. C. (1987) *Nature (London)* **327,** 518–522.
9. Robb, R. J. & Greene, W. C. (1987) *J. Exp. Med.* **165,** 1201–1206.
10. Wang, H.-M. & Smith, K. A. (1987) *J. Exp. Med.* **166,** 1055–1069.
11. Ju, G., Collins, L., Kaffka, K. L., Tsien, W.-H., Chizzonite, R., Crowl, R., Bhatt, R. & Kilian, P. L. (1987) *J. Biol. Chem.* **262,** 5723–5731.
12. Bernard, H. & Helinski, D. R. (1979) *Methods Enzymol.* **68,** 482–492.
13. Hakimi, J., Seals, C., Anderson, L. E., Podlaski, F. J., Lin, P., Danho, W., Jenson, J. C., Perkins, A., Donadio, P., Familletti, P. C., Pan, Y.-C. E., Tsien, W.-H., Chizzonite, R. A., Casabo, L., Nelson, D. L. & Cullen, B. R. (1987) *J. Biol. Chem.* **262,** 17336–17341.
14. Bailon, P., Weber, D. V., Keeney, R. F., Fredericks, J. E., Smith, C., Familletti, P. C. & Smart, J. E. (1987) *Bio/Technology* **5,** 1195–1198.
15. Kuo, L.-M. & Robb, R. J. (1986) *J. Immunol.* **137,** 1538–1543.
16. Robb, R. J., Kutny, R. M., Panico, M., Morris, H. R. & Chowdhry, V. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6486–6490.
17. Arakawa, T., Boone, T., Davis, J. M. & Kenney, W. (1986) *Biochemistry* **25,** 8274–8277.
18. Liang, S.-M., Thatcher, D. R., Liang, C.-M. & Allet, B. (1986) *J. Biol. Chem.* **261,** 334–337.
19. Liang, S.-M., Lee, N., Zoon, K. C., Manischewitz, J. F., Chollet, A., Liang, C.-M. & Quinnan, G. (1988) *J. Biol. Chem.* **263,** 4768–4772.
20. Brandhuber, B. J., Boone, T., Kenney, W. C. & McKay, D. B. (1987) *Science* **238,** 1707–1709.