Biochem. J. (1992) 285, 851–856 (Printed in Great Britain)

Characterization of a tyrosine kinase activity associated with the high-affinity interleukin 2 receptor complex

Gonzalo G. GARCIA,* Gerald A. EVANS,* Dennis F. MICHIEL,† and William L. FARRAR†‡
*Biological Carcinogenesis and Development Program, Program Resources, Inc./DynCorp,
NCI–Frederick Cancer Research and Development Center, and †Laboratory of Molecular Immunoregulation,
Biological Response Modifiers Program, National Cancer Institute, Frederick, MD 21702-1201, U.S.A.

The IL-2 receptor complex is minimally composed of two genetically unrelated subunits of relative molecular masses 55 and 75 kDa respectively. Structural information deduced from the cDNA sequences of either subunit have not revealed significant information as to the basis of the mechanisms of IL-2 receptor signal transduction. Nevertheless, IL-2 stimulates the activation of one or more tyrosine kinases requiring the functional participation of the p75 member of the receptor complex. Here we have developed the methods to isolate the receptor complex with an associated tyrosine protein kinase. Extracts of membrane glycoproteins from activated normal human T lymphocytes and cell lines demonstrated catalytic activation of tyrosine kinase activity when stimulated with IL-2. Purification of the receptor complex with biotinylated IL-2 revealed the presence of two dominant phosphotyrosyl-proteins of approximate molecular masses 58 and 97 kDa. Denaturation gel electrophoresis followed by renaturation of proteins associated with the IL-2 receptor complex demonstrated that the 97 kDa protein had catalytic autophosphorylation activity. The results indicate that the 58 and 97 kDa phosphotyrosyl-proteins can be found to co-precipitate with the IL-2 receptor complex and that the 97 kDa protein was demonstrated to have protein kinase activity. The association of such kinases with receptors devoid of catalytic structure may represent a unique paradigm of growth-factor receptor mechanisms.

INTRODUCTION

The p75 β-chain glycoprotein, which is a constituent of the high-affinity interleukin 2 receptor (IL-2R) complex is a member of a new family of receptor/recognition molecules termed the 'haematopoietin superfamily'. Receptor proteins included in this superfamily recognize diverse ligands, including tumour necrosis factor, IL-3, IL-4, IL-7, granulocyte macrophage colony-stimulating factor, erythropoietin and the endocrine hormone prolactin [1–3]. Common gene organizations exist among the members of this family, and the most astonishing feature is the finding that no members of this family have any apparent catalytic domains. This fact appears structurally to distinguish members of this family from other well-studied receptors for growth factors such as epidermal growth factor, platelet-derived growth factor and insulin, which appear to transduce membrane signalling by the activation of intrinsic tyrosine kinase activity [4]. Nevertheless, cytokine ligands, including IL-2, activate tyrosine kinase activity [5–8]. Since both the known subunits of the interleukin 2 receptor (IL-2R) complex have no structural domains showing sequence similarities to features characteristic of tyrosine kinases or other enzyme activities [9,10], it appears that the regulation of tyrosine kinases by IL-2 stimulation may use unique mechanisms distinct from that observed with other growth-factor receptors.

Initially, IL-2 was shown to increase tyrosine phosphorylation of a number of proteins detected by one-dimensional SDS/PAGE and immunoblotting with anti-phosphotyrosine antibody [5]. Workers in our laboratory further studied the regulation of tyrosine phosphorylation by IL-2 stimulation using high-resolution two-dimensional electrophoresis and phosphoamino acid analysis. We demonstrated that IL-2 stimulates tyrosine phosphorylation on a number of proteins ranging from 180 to 43 kDa [6,7]. The same or a similar set of proteins are phosphorylated upon IL-2 stimulation of normal human T lymphocytes, which express both the p55 IL-2Rα and p75 IL-2Rβ subunits, and leukaemic cell lines, which express only the p75 IL-2Rβ chains [6]. Furthermore, normal human large granular lymphocytes, which express only the p75 IL-2Rβ chain, respond to IL-2 by phosphorylation of the same proteins found in proliferating lymphocytes, where both chains participate to form the high-affinity complex [7]. Thus the signal-transduction process which results in tyrosine kinase activation appears to require only the participation of the p75 subunit of the complex, the p55 chain appearing to function primarily in the regulation of the ligand affinity state.

We have developed a unique methodology which has allowed us to extract the IL-2R complex and stimulate the activation of an associated tyrosine protein kinase *in vitro* with the physiological ligand. The p55 and p75 glycoproteins of the IL-2R complex can be extracted from activated human T cells and cell lines with detergent and bind avidly to lectins such as concanavalin A (Con A) and wheat-germ agglutinin. After elution from lectin–Sepharose with the appropriate sugar, ligand binding to both subunits can be demonstrated using ^{125}I-IL-2 [11]. The addition of IL-2 and ATP revealed the presence of tyrosine protein kinase activity observed by increased phosphorylation of proteins detected by anti-phosphotyrosine monoclonal antibody (mAb). Furthermore, using biotinylated IL-2 (b-IL-2)–streptavidin–agarose we precipitated the IL-2R complex and found at least two major phosphotyrosyl-proteins associated with the complex. Renaturation of protein kinase activity within

Abbreviations used: rhIL-2, recombinant human interleukin 2; b-IL-2, biotinylated interleukin 2; b-GM-CSF, biotinylated granulocyte macrophage colony-stimulating factor; Con A, concanavalin A; mAb, monoclonal antibody; IL-2R, interleukin 2 receptor; PMSF, phenylmethanesulphonyl fluoride; NEPHGE, non-equilibrating pH gel electrophoresis.

‡ To whom correspondence should be sent, at the following address: Cytokine Molecular Mechanisms Section, Laboratory of Molecular Immunoregulation, National Cancer Institute, Building 560, Room 21-89A, Frederick, MD 21702-1201, U.S.A.

the IL-2R complex showed a 97 kDa protein to have catalytic autophosphorylation properties. These data suggested that the 97 kDa protein may be a protein kinase important for the IL-2R signal-transduction process.

MATERIALS AND METHODS

YT cells (human NK-like cell line expressing large amounts of p70–75 relative to p55) and MLA-144 cell line were grown and prepared as described elsewhere [6,13]. Human T lymphocytes were purified from human peripheral-blood mononuclear cells and activated as described previously [7,11].

Preparation of the IL-2R extracts and *in vitro* protein kinase assays

Cells were solubilized at 4 °C for 90 min in 1% Nonidet P40/50 mM-Hepes (pH 7.2)/150 mM-NaCl/5 mM-EDTA/1 mM-phenylmethanesulphonyl fluoride (PMSF)/soybean trypsin inhibitor (0.2 mg/ml)/leupeptin (2 μg/ml)/pepstatin A (2 μg/ml)/100 μM-sodium orthovanadate/1% BSA. After centrifugation, the IL-2R complex was partially purified by chromatography on Con A–Sepharose 4B (Pharmacia; Uppsala, Sweden) as described by Michiel *et al.* [11]. The Con A-bound receptor complex was eluted with 0.6 M-α-methyl D-mannopyranoside in kinase buffer (0.1% Nonidet P40 in 50 mM-Hepes, pH 7.2, containing 150 mM-NaCl, 10 mM-$MgCl_2$, 10 mM-$MnCl_2$, 100 μM-sodium orthovanadate, 100 μM-dithiothreitol and 10% (v/v) glycerol).

For immunoprecipitations with PY20 anti-phosphotyrosine mAb (ICN, Biochemicals, Cleveland, OH, U.S.A.) [12], 150 μg of protein eluted from Con A–Sepharose was incubated in presence or absence of 10 μg of recombinant human IL-2 (rhIL-2) for 20 min at room temperature. Phosphorylation was initiated by the addition of 150 μCi of [γ-^{32}P]ATP (3000 Ci/mmol; du Pont–New England Nuclear, Boston, MA, U.S.A.). After incubation for 5 min at 30 °C the reaction was stopped by the addition of 100 mM-EDTA and 10 mM-ATP. Phosphotyrosyl-proteins were immunoprecipitated with PY-20 mAb, eluted with phenol phosphate after extensive washing, and analysed by non-equilibrating pH gel electrophoresis (NEPHGE)–SDS/PAGE two-dimensional electrophoresis as previously described [6].

Purification of IL-2R complex with b-IL-2

rhIL-2 (Hoffman–La Roche, Nutley, NJ, U.S.A.) or recombinant human granulocyte macrophage colony-stimulating factor (rhGM-CSF; Boehringer Mannheim Biochemicals, Indianapolis, IN, U.S.A.) were biotinylated by the method of Taki *et al.* [14], with the modification described by Michiel *et al.* [11].

For additional purification of the IL-2R complex, 150 μg of the Con A–Sepharose eluate was incubated with 10 μg of rhIL-2 (or GM-CSF) or 10 μg of b-IL-2 (or GM-CSF) for 20 min at room temperature. Phosphorylation was performed as described above and the reaction mixtures were incubated with streptavidin–agarose for 1 h at 4 °C. After washing with 50 mM-Hepes (pH 7.2)/150 mM-NaCl/0.1% Nonidet P40/10% glycerol (Hepes buffer), the IL-2R complex was eluted with citrate buffer, pH 3.5, containing 0.1% Nonidet P40 and analysed by two-dimensional electrophoresis.

Denaturation–renaturation kinase and '*in vitro*' tyrosine kinase–substrate assays

For analysis of kinase activity found in association with the purified IL-2R complex, 120 μg of the Con A–Sepharose extracts from YT cells was incubated with 10 μg of IL-2 or b-IL-2. The mixture was then incubated with streptavidin–agarose for 1 h at 4 °C. The streptavidin–agarose was washed with Hepes buffer, followed by kinase buffer. Phosphorylation was performed in kinase buffer with 20 μCi of [γ-^{32}P]ATP for 20 min at 30 °C. The reaction was stopped with 100 mM-EDTA/10 mM-ATP, washed with Hepes buffer, then eluted with citrate buffer, pH 3.8, and analysed by two-dimensional electrophoresis.

The '*in vitro*' tyrosine kinase–substrate assay was carried out on extracts purified from Con A–Sepharose or biotinylated lymphokines (IL-2 or GM-CSF) as described above with the addition of synthetic substrates. The phosphorylation reaction mixtures were incubated for 5 min at 30 °C in the presence or absence of tyrosine synthetic substrates, poly[Glu,Tyr(4:1)] (Sigma, St. Louis, MO, U.S.A.) or Src peptide (Bachem, Torrance, CA, U.S.A.) (2 mg/ml) and aliquots (5 μl) were spotted on Whatman P-81 paper (2 cm × 2 cm). The papers were washed with 0.5% phosphoric acid containing 1 mM-sodium pyrophosphate, dried, and ^{32}P incorporation was determined by liquid-scintillation counting.

In the denaturation–renaturation assay the IL-2R complex was purified from T cells by using the Con A–Sepharose procedure, followed by b-IL-2–streptavidin–agarose and eluted from the precipitate with citrate buffer, pH 3.8. The samples were resolved by SDS/PAGE and transferred to Immobilon poly(vinylidene difluoride) membranes (Millipore, Bedford, MA, U.S.A.). The proteins were renatured and kinase activity assayed by the method of Ferrel & Martin [15].

Phosphoamino acid analysis

Two-dimensional gels containing b-IL-2–streptavidin–agarose-purified IL-2R complex proteins phosphorylated *in vitro* as previously described were electrophoretically transferred to Immobilon and the p97/p58 bands were excised and acid-hydrolysed with 5 M-HCl at 110 °C for 2 h. The hydrolysate was mixed with a pool of purified standard phosphoamino acids (phosphoserine, phosphothreonine and phosphotyrosine; Sigma) and separated by h.p.l.c. as described by Chang *et al.* [16]. To detect incorporation of ^{32}P the phosphoamino acids eluted from the h.p.l.c. column were collected, dried by speed vacuum and resuspended in 10 μl of water; samples were then spotted on Whatman 3 MM paper, dried and autoradiographed.

RESULTS

Glycoprotein extracts were prepared from the cells which express both the p55 and p75 subunits or only the p75 protein. These glycoprotein extracts, containing the receptor complex, retained the ability to bind IL-2 to both α- and β-subunits [11]. Tyrosine protein kinase activity was detected upon the addition of IL-2 and ATP to a receptor-extract kinase reaction mixture, and phosphotyrosyl-proteins were revealed by immunoprecipitation with anti-phosphotyrosine mAb PY20 [12], followed by two-dimensional NEPHGE. Fig. 1 shows the autoradiographs of phosphotyrosyl-proteins regulated in receptor extracts where IL-2 was added. In the YT cell line, which expresses higher amounts of the p75 protein compared with p55 [13], the addition of IL-2 stimulated the tyrosine phosphorylation of several proteins, with the dominant proteins found at approx. 97 and 55–58 kDa. Proteins with the same mass and charge (pI 5.3) were found to be phosphorylated in receptor extracts prepared from activated human T cells (p55 and p75 chain) and the gibbon leukaemia cell line MLA-144, which expresses only the p75 chain [6]. Unstimulated receptor extracts had very few detectable phosphotyrosyl-proteins. Phosphoamino acid analysis using h.p.l.c. of the proteins eluted from the gels at the 97 kDa and 58 kDa positions confirmed that the phosphoproteins precipitated by the anti-

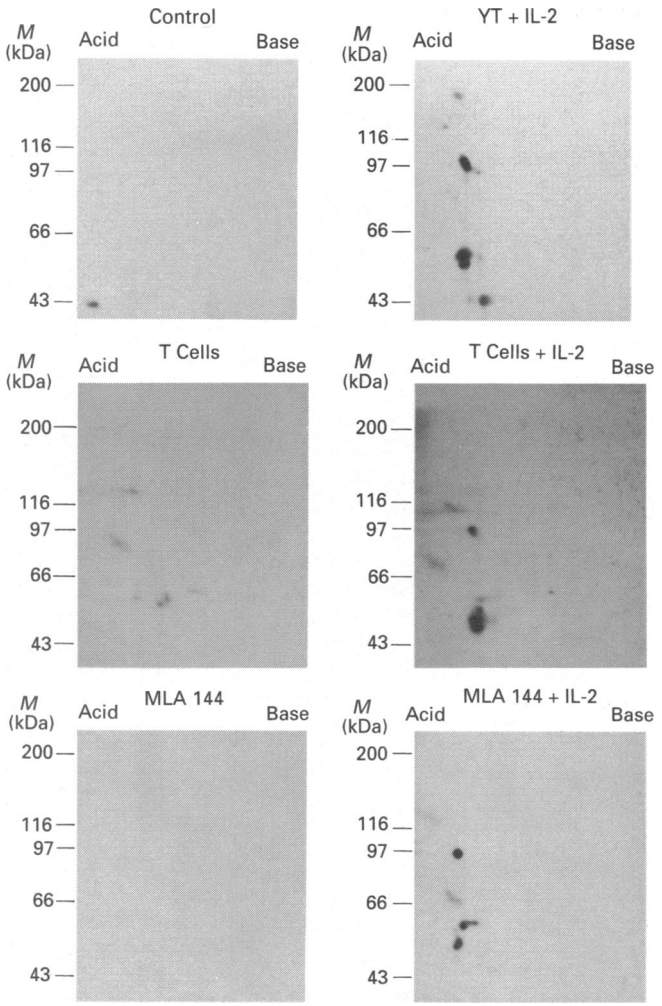

Fig. 1. *In vitro* tyrosine kinase activity of glycoprotein extracts stimulated by IL-2

YT cells, activated human T cells and MLA-144 cells were grown and prepared as described in the Materials and methods section. Cells were solubilized and the IL-2 receptor complex partially purified by chromatography on Con A–Sepharose 4B. Protein kinase activity in the Con A–Sepharose eluate was assayed in the absence (Control) or presence of 10 μg of rhIL-2 (+IL-2). Phosphotyrosyl-proteins were immunoprecipitated with PY-20 mAb, eluted with phenyl phosphate and analysed by NEPHGE–SDS/PAGE two-dimensional electrophoresis. Autoradiographs show the effect of IL-2 stimulation on the phosphorylation of specific substrates detected in each cell line extract. Abbreviation: *M*, molecular mass.

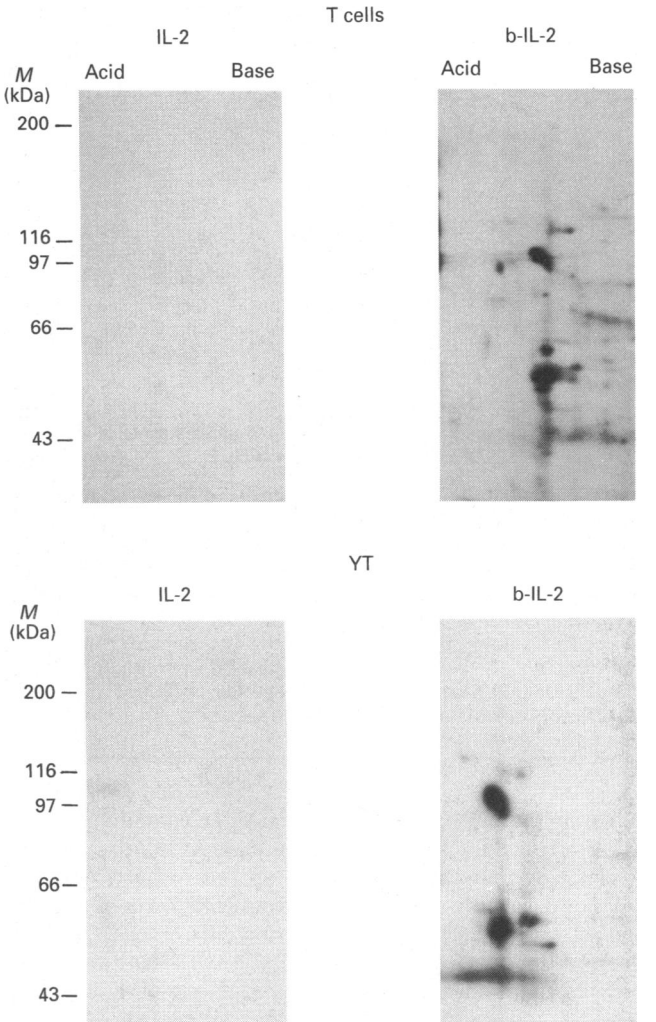

Fig. 2. Two-dimensional analysis of phosphoproteins associated with the IL-2 receptor complex purified by ligand affinity chromatography using b-IL-2

The eluate from Con A–Sepharose chromatography of either activated-T-cell (upper panels) or -YT-cell (lower panels) extracts was phosphorylated *in vitro* by the addition of [^{32}P]ATP in the presence of unconjugated IL-2 (left panels) as a control or b-IL-2 (right panels). The b-IL-2–IL-2R complex was then precipitated with streptavidin–agarose and analysed by NEPHGE–SDS/PAGE two-dimensional electrophoresis. Abbreviation: *M*, molecular mass.

phosphotyrosine mAb were indeed phosphorylated on tyrosine residues (results not shown). Additional phosphotyrosyl-proteins were also seen, including one at approx. 70–75 kDa found in the T-cell and MLA-144 glycoprotein extracts. This protein may be the p75 β-chain, since it is known to be phosphorylated by IL-2 stimulation [8]. The high degree of constitutive phosphorylation of the p75 chain found in the YT cell line *in situ* [13] may make *in vitro* phosphorylation of sites already modified *in situ* more difficult to reveal.

These data suggested that the addition of IL-2 to receptor extracts from cells containing either the p55 and p75 subunits or p75 subunit alone resulted in the activation of one or more tyrosine kinases, as indicated by the tyrosine phosphorylation of several substrates. In order to ascertain which of these phosphoproteins may be intimately associated with the receptor complex itself, we used b-IL-2 as an affinity probe for precipitating the receptor complex. b-IL-2 has previously been shown to show stable biological activity and affinity for both receptor subunits [14]. Fig. 2 shows the phosphoproteins associated with the receptor complex purified by streptavidin–agarose precipitation of the b-IL-2–IL-2R complex after the *in vitro* kinase assay. In these experiments unconjugated IL-2 served as the control for the streptavidin–agarose precipitation. IL-2, biotin or b-GM-CSF alone was unable to serve as a precipitating ligand with extracts eluted from Con A–Sepharose from either normal activated human T lymphocytes or YT cells. However, the b-IL-2 was capable of precipitating phosphoproteins at the 97 and 58 kDa. These proteins had a mobility identical with that of those found using the anti-phosphotyrosine mAb after the *in vitro* kinase assay (Fig. 1). Other minor phosphoproteins were also observed in the purified receptor b-IL-2 precipitations, but the 97 and 58 kD proteins were clearly the most abundantly phosphorylated. Often the 97 and 58 kDa proteins focused as

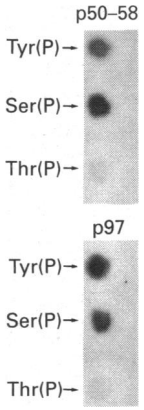

Fig. 3. Phosphoamino acid analysis of p97 and p55–58 proteins associated with the IL-2R complex purified by b-IL-2

IL-2R complex from YT cells was purified and the kinase reaction performed as described in Fig. 2. The samples were analysed by NEPHGE–SDS/PAGE two-dimensional electrophoresis and transferred to Immobilon membranes. The phosphoproteins were identified by autoradiography and p97/p55–58 was excised, hydrolysed and the phosphoamino acids separated by h.p.l.c. The ^{32}P incorporated into phosphoamino acids was revealed by autoradiography of the phosphoserine [Ser(P)], phosphothreonine [Thr(P)] or phosphotyrosine [Tyr(P)] spotted on 3 MM paper as described in the Materials and methods section.

doublets in the anti-phosphotyrosine mAb precipitations and the b-IL-2–streptavidin–agarose precipitations. At this time we cannot conclude that these doublets represent distinct molecular species or covalently modified phosphorylated forms of the same principal proteins. However, the minor differences in mobility reflected by the appearance of doublets is consistent with many molecules that undergo multiple sites of phosphorylation.

The 97 and 58 kDa proteins were excised from the two-dimensional gels, which resolved the b-IL-2–IL-2R complex precipitated in the *in vitro* kinase reaction (Fig. 2), amino acid hydrolysis was done and the residues were separated by h.p.l.c. and analysed (Fig. 3). The results showed that both the 97 and 58 kDa proteins had tyrosine and serine residues phosphorylated. These experiments showed that the addition of IL-2 to *in vitro* glycoprotein extracts could stimulate tyrosine phosphorylation of specific substrates which were found in association with the receptor complex.

In order to determine whether any of the phosphoproteins observed in association with the receptor complex had catalytic kinase activity, we precipitated the receptor complex with b-IL-2 first and then added ATP to initiate the *in vitro* kinase assay. Fig. 4(*a*) shows the results of a typical experiment in which kinase activity was observed in b-IL-2 precipitations. Although a number of phosphoproteins were seen, the dominant proteins were identical in molecular mass and pI with the 97 and 58 kDa phosphotyrosyl-proteins observed with all the other *in vitro* kinase assay designs. The control receptor purification with unconjugated IL-2 did not reveal any kinase activity, as indicated by the lack of substrate phosphorylation. The receptor complexes purified from T cells and the YT cell line with biotinylated IL-2 were further tested for tyrosine kinase activity by adding the receptor complexes *in vitro* kinase reaction mixtures containing the synthetic tyrosine kinase substrates Src peptide or poly[Glu,Tyr(4:1)]. b-GM-CSF was used as a ligand control. As Fig. 4(*b*) shows, b-IL-2 precipitated tyrosine kinase activity detected by synthetic substrate phosphorylation. The poly[Glu,Tyr(4:1)] substrate tended to be preferred, and the YT cell line, which has higher concentrations of receptor subunits,

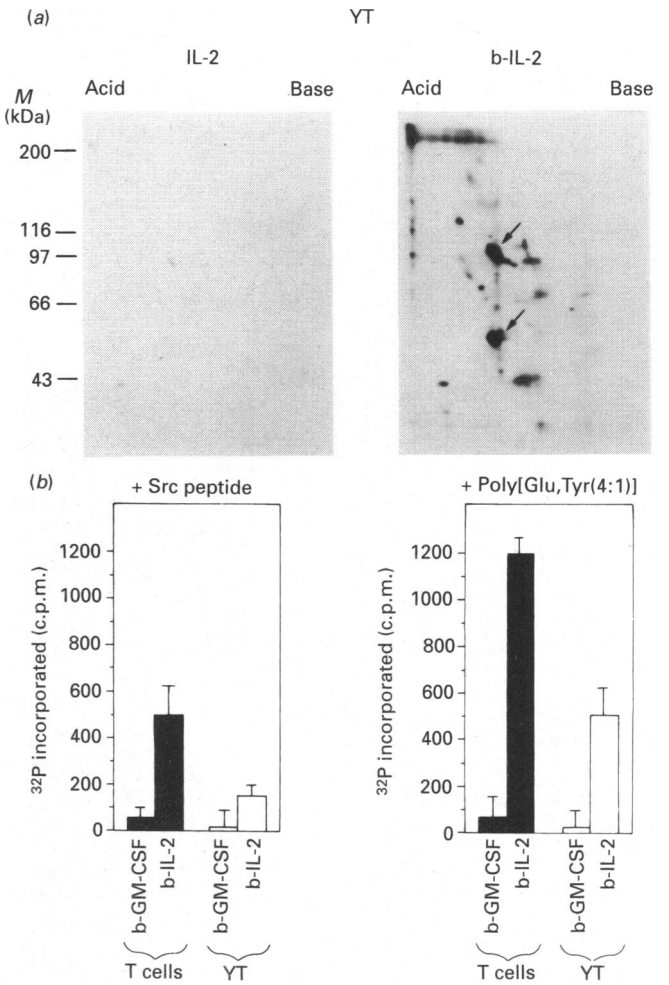

Fig. 4. Ligand-affinity-purified IL-2R complex expresses tyrosine kinase activity

Eluate from Con A–Sepharose chromatography of YT-cell extract (120 μg) (*a*) was incubated with 10 μg of IL-2 (left panel) or b-IL-2 (right panel), then with streptoavidin–agarose for 1 h at 4 °C. After washing, phosphorylation was carried out as described in the Materials and methods section. The reaction was stopped with 100 mM-EDTA/10 mM-ATP. Associated proteins were eluted with citrate buffer, pH 3.8, and analysed by NEPHGE–SDS/PAGE two-dimensional electrophoresis. The tyrosine kinase activity within the IL-2R complex was identified by phosphorylation of synthetic tyrosine kinase substrates (*b*). b-GM-CSF was used as a control. The kinase reaction was run in the presence of Src peptide (left panel) or poly[Glu,Tyr(4:1)] (right panel). Values are averages ± S.D. for ^{32}P incorporation data from four different experiments. The b-GM-CSF does not show a significant tyrosine kinase activity.

gave higher tyrosine kinase catalytic activity in the purified receptor extracts. These experiments further confirmed that a tyrosine kinase catalytic activity could be detected in purified IL-2R complexes. The foregoing experiments revealed that tyrosine kinase activity could be found in association with ligand-affinity-purified IL-2 receptor complex extracts and further suggested that either of the two major phosphotyrosyl-proteins found might have kinase activity. The design of the *in vitro* receptor kinase assay does not distinguish between autophosphorylated tyrosine protein kinases and substrates, since both sets of proteins will be phosphorylated on tyrosine residues. We next used a procedure which renatures proteins from SDS/PAGE and which allowed us to determine the intrinsic catalytic activity of proteins associated in the IL-2R complex represented by autophosphorylation. Receptor complexes from activated human T cells were

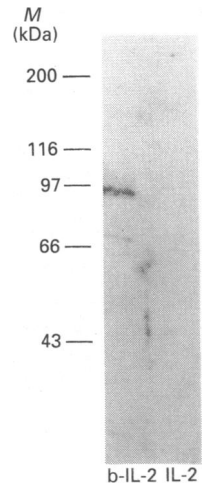

Fig. 5. IL-2R complex purified with b-IL-2–streptavidin–agarose contains a p97 protein with autophosphorylation kinase activity

The IL-2R complex was purified from T cells using the Con A–Sepharose and b-II-2–streptavidin–agarose procedures described in the Materials and methods section. The complex was eluted from the streptavidin–agarose, subjected to SDS/PAGE and transferred to Immobilon membrane; the proteins were renatured and kinase activity was assayed; the autoradiograph shows the autophosphorylation of p97. Kinase activity is absent when unmodified IL-2 is used as a control purification ligand ('IL-2').

purified by b-IL-2–streptavidin–agarose precipitation of glycoprotein extracts eluted from Con A–Sepharose. The protein samples were first denatured and separated by SDS/PAGE. The gel proteins were then transferred to Immobilon membrane, renatured and ATP added. This assay detects autophosphorylating protein kinase activity (Fig. 5) [15]. A single 97 kDa protein was consistently found to be autophosphorylated in the purified IL-2 receptor complexes obtained from activated normal human T cells. Similar data was seen with receptor complexes isolated from the YT cell line. Although the catalytic renaturation experiment was performed by using SDS/PAGE, we were unable to renature any catalytic activity when using two-dimensional procedures in the presence of urea. Nevertheless, since most of our *in vitro* kinase-IL 2 receptor complex assays revealed only one major phosphotyrosyl-protein at approx. 97 kDa, we feel that the catalytic activity detected in the renaturation experiment is most likely the 97 kDa phosphotyrosyl-protein seen in all the other IL-2R *in vitro* kinase assays.

DISCUSSION

We have developed the techniques to study *in vitro* the activation of protein kinases associated with the IL-2R system. The multi-unit structure of the proteins regulating ligand affinity states and proteins involved with signal transduction represent a highly complex biochemical mechanism in controlling the biological response to IL-2. Whereas the proteins which govern the affinity states of the IL-2R have been rigorously studied, almost nothing is known about the proteins which associate with the receptor complex that have any known catalytic function. Although a number of additional subunits have been proposed and observed, particularly in the murine IL-2R complex [17,18], we do not know whether any of the additional proteins found within our receptor precipitations are related to any of the additional subunits proposed by others. A lack of specific antibodies against subunits other than the p55 and p75 chains make difficult assumptions as to the relatedness of our observations to other putative subunits. Here we have shown that at least two dominant phosphotyrosyl-proteins are associated with the high-affinity IL-2R complex and may be uniquely associated with the p75 subunit. Our findings that the same proteins are associated with either affinity states governed by the presence of p55 and p75 is not surprising, since the p75 chain alone is capable of initiating tyrosine kinase activation and signals leading to gene expression. Ligand affinity purification of the receptor complex detected an associated tyrosine protein kinase activity which, upon activation by the ligand, resulted in the phosphorylation of several substrates. One of these substrates, a 97 kDa phosphotyrosyl-protein, could be demonstrated to have catalytic protein kinase activity and was in physical association with the IL-2R complex.

We cannot determine with certainty whether the 58 kDa protein is not a kinase; rather our data may reflect its inability to renature catalytic activity under the conditions used. Thus whether additional protein kinases are associated with the IL-2R complex, such as the src-like protein kinases found to cluster with the platelet-derived-growth-factor receptor [19], remains an issue for further investigation. In this regard a recent report by Hatakeyama *et al.* [20] demonstrated the $p56^{lck}$ could be found associated with p75 precipitated with αp75 mAb. However, transient transfection studies revealed that the $p56^{lck}$ did not associate with the *C*-terminal region of p75 required for IL-2-induced proliferation. Furthermore, BAF-3 cells transfected with p75 proliferate in response to IL-2 in the absence of detectable $p56^{lck}$ [21], and CTLL-2 cells not expressing $p56^{lck}$ also proliferate in response to IL-2 (W. L. Farrar, unpublished work). These data support the notion that another kinase is probably the principal signal enzyme. The identity of the 58 kDa and 97 kDa phosphotyrosyl-proteins are unknown. We have immunoblotted this region of the gel with antisera against LCK peptides, src-COOH peptide, anti-LTK, and anti-FES peptide and have not found any immunoreactive protein associated with the b-IL-2 precipitated material. Although we have not blotted the b-IL-2 precipitated material with antibody to $p56^{fyn}$, we cannot entirely exclude all members of the src family of protein kinases from potential association with the IL-2R complex, even though anti-(src-COOH peptide) antibody did not recognize the 58 kDa proteins. Furthermore, non of these antisera were capable of precipitating ^{125}I-IL-2 cross-linked to YT cells (results not shown). The 97 kDa catalytic protein may represent a unique kinase unrelated to the src family or other families recently described [22,23], although further purification is necessary for a better understanding of the function of this enzyme.

The activation of protein kinases and particularly tyrosine protein kinases has been obligatory for the biological action of many growth factors. The identification of a protein kinase associated with a member of a new family of growth-factor receptors may constitute a new mechanistic paradigm for growth-factor receptors where catalytic activities are not intrinsic to the structure of the principal ligand-binding protein. Our finding of a tyrosine protein kinase within the IL-2R complex suggests that this kinase may be of fundamental importance to the signal-transduction process initiated by a receptor system fundamental to T-cell growth.

We thank Dr. John O'Shea, Dr. Joost J. Oppenheim and Dr. Dan Longo for useful comments and support. We are also grateful to Dr. Joe Bolen and Bob Glazer for providing the anti-(src family) antisera. This project was funded in part with Federal funds from the Department of Health and Human Services under contract no. N01-CO-74102 with Program Resources Inc. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products or organizations imply endorsement by the U.S. Government.

REFERENCES

1. Bazan, J. F. (1989) Biochem. Biophys. Res. Commun. **164**, 788–795
2. D'Andrea, A. D., Fasman, G. D. & Lodish, H. F. (1989) Cell (Cambridge, Mass.) **58**, 1023–1024
3. Goodwin, R. G., Friend, D., Ziegler, S. F., Jerzy, R., Falk, B. A., Gimpel, S., Cosman, D., Dower, S. K., March, C. J., Namen, A. E. & Park, L. S. (1990) Cell (Cambridge, Mass.) **60**, 941–951
4. Ullrich, A. & Schlessinger, J. (1990) Cell (Cambridge, Mass.) **61**, 203–212
5. Saltzman, E. M., Thom, R. R. & Casnellie, J. E. (1989) J. Biol. Chem. **263**, 6956–6959
6. Farrar, W. K. & Ferris, D. K. (1989) J. Biol. Chem. **264**, 12562–12567
7. Ferris, D. K., Willette-Brown, J., Ortaldo, J. R. & Farrar, W. L. (1989) J. Immunol. **143**, 870–876
8. Hironobo, A., Toshikazu, T., Masatara, N., Kinya, N. & Kazuo, S. (1990) J. Exp. Med. **171**, 637–644
9. Leonard, W. J., Depper, J. M., Crabtree, G. R., Rudikoff, S., Pumphrey, J., Robb, R. J., Kronke, M., Svetlik, P. B., Peffer, N. J., Waldmann, T. A. & Greene, W. C. (1984) Nature (London) **311**, 626–631
10. Hatakeyama, M., Tsudo, M., Minamoto, S., Kono, T., Doi, T., Miyata, T., Miyasaka, M. & Taniguichi, T. (1989) Science **244**, 551–556
11. Michiel, D. F., Garcia, G. G., Evans, J. A. & Farrar, W. L. (1991) Cytokine **3**, 428–438
12. Glenney, J. R., Jr., Zokas, L. & Kamps, M. P. (1988) J. Immunol. Methods **109**, 277–285
13. Sharon, M., Gnarra, J. R. & Leonard, W. J. (1989) J. Immunol. **143**, 2530–2533
14. Taki, S., Shimamura, T., Abe, M., Shirai, T. & Takahara, Y. (1989) J. Immunol. Methods **122**, 33–41
15. Ferrell, J. E., Jr. & Martin, G. S. (1989) J. Biol. Chem. **264**, 20723–20729
16. Chang, J. Y. (1984) J. Chromatogr. **295**, 193–200
17. Saragovi, H. & Malek, T. R. (1990) Proc. Natl. Acad. Sci. **87**, 11–15
18. Sharon, M., Gnarra, J. & Leonard, W. J. (1989) Clin. Res. **37**, 989
19. Kyptu, R. M., Goldberg, Y., Ulug, E. T. & Courtneidge, S. A. (1990) Cell (Cambridge, Mass.) **62**, 481–492
20. Hatakeyama, M., Kono, T., Kabayashi, N., Kawahara, A., Levin, S. D., Perlmutter, R. M. & Taniguchi, T. (1991) Science **252**, 1523–1528
21. Hatakeyama, M., Mori, H., Doi, T. & Taniguchi, T. (1989) Cell (Cambridge, Mass.) **59**, 837–845
22. Ben-David, Y., Letwin, K., Tannock, L., Bernstein, A. & Pawson, T. (1991) EMBO J. **10**, 317–325
23. Howell, B. W., Afar, D., Lew, J., Douville, E., Icely, P., Gray, D. & Bell, J. (1991) Mol. Cell. Biol. **11**, 568–572

Received 14 October 1991/14 January 1992; accepted 4 February 1992