Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 1243–1246, February 1990
Biochemistry

Photoaffinity labeling of ATP and NAD^+ binding sites on recombinant human interleukin 2

(signal transduction/autophosphorylation/glycoprotein hormone/lymphokine)

SUSAN CAMPBELL*, HYUNTAE KIM*, MICHAEL DOUKAS†, AND BOYD HALEY*

Departments of *Biochemistry †Medicine and the Lucille Parker-Markey Cancer Center, University of Kentucky, 800 Rose Street, Lexington, KY 40536

Communicated by Paul D. Boyer, November 30, 1989

ABSTRACT **Interleukin 2 (IL-2) is a T-cell-derived lymphokine critical in the activation and proliferation of T cells, B cells, and lymphokine-activated killer cells. It is a glycoprotein of ≈15,500 daltons that is synthesized and secreted after activation by antigen or mitogen. By using the analogs 8-azidoadenosine 5′-[γ-^{32}P]triphosphate ([γ-^{32}P]8N_3ATP) and nicotinamide 2-azidoadenine [*adenylate*-^{32}P]dinucleotide ([α-^{32}P]2N_3NAD^+) as photoaffinity probes, we have detected specific, metal ion-requiring nucleotide binding sites on recombinant human IL-2 (rhIL-2). The specificity of these nucleotide interactions with rhIL-2 was demonstrated by saturation effects and by competition by the parent nucleotides at physiologically relevant concentrations. Saturation of photoinsertion into rhIL-2 occurred at 50 μM [γ-^{32}P]8N_3ATP; a half-maximal decrease of its photoinsertion at 10 μM was obtained with 22 μM ATP. Saturation of photoinsertion with [α-^{32}P]2N_3NAD^+ was observed at 180 μM; a half-maximal decrease of its photoinsertion at 10 μM was effected by 10 μM NAD^+ and by 5 μM 3-aminobenzamide. The extent of photoinsertion of both photoprobes into rhIL-2 varied with the presence of different divalent metal ions. rhIL-2 photolabeling with [γ-^{32}P]8N_3ATP appeared to be dependent on the presence of metal ion. It was effectively labeled in the presence of Mg^{2+} and photoinsertion was increased with the addition of Zn^{2+} at micromolar concentrations. Also, rhIL-2 underwent slow autophosphorylation by an intramolecular mechanism using [γ-^{32}P]8N_3ATP as well as nonphotoactive nucleotide. The biological significance of these interactions is unknown, but their specificity suggests that nucleotide binding may be involved in the bioactivity of IL-2.**

Interleukin 2 (IL-2), also called T-cell growth factor, is a glycosylated polypeptide growth hormone discovered by Morgan *et al.* (1). IL-2 is produced upon antigen or mitogen stimulation of T lymphocytes and is necessary for the proliferation and stimulation of the effector function of activated T cells (2), natural killer cells (3–5), and other cytotoxic effector cells (6–8). Additionally, IL-2 interacts with B cells and macrophages and elicits or enhances production of other cytokines such as colony-stimulating factors, B-cell growth factors, and interferon (9). Thus, IL-2 plays a critical role in regulation of the immune system and indirectly affects differentiation and hematopoietic cell growth. Robb *et al.* (10) used radiolabeled IL-2 to show that IL-2-dependent cells had two distinct classes of IL-2 receptors, each with a distinct affinity for radiolabeled IL-2. Evidence suggests that the high-affinity class of IL-2 receptor is the class of sites that correlates with the minimum IL-2 concentration required for proliferation of T cells (11) and that only the high-affinity IL-2 receptors are internalized upon binding IL-2 (12, 13). Data presented by Evans *et al.* (14) suggest that the IL-2 receptor mediates signal transduction via a GTP-binding protein. However, the exact intracellular biochemical pathways activated by IL-2 binding to its receptor have not been clearly elucidated.

Human IL-2 is produced by helper T cells in minute quantities, but the development of high-producer tumor cell lines like the human Jurkat leukemic line has permitted the purification of human IL-2 to homogeneity and the determination of its amino acid sequence (15). Cloned cDNA for human IL-2 has been obtained from a number of cell lines (16–19), and the IL-2 cDNA sequence has been found to code for a polypeptide chain of 153 amino acids, including a 20-amino acid putative signal sequence. A biologically active recombinant human IL-2 (rhIL-2) molecule of 133 amino acids and a molecular mass of 15,000 daltons has been obtained by expression of these cDNA clones in *Escherichia coli* (17) and cultured monkey COS cells (16). The rhIL-2 and a highly purified lymphocyte IL-2 have been shown to have nearly identical biological properties (18). Utilizing the photoaffinity probes 8-azidoadenosine 5′-[γ-^{32}P]triphosphate ([γ-^{32}P]8N_3ATP) and oxidized nicotinamide 2-azidoadenine [*adenylate*-^{32}P]dinucleotide ([α-^{32}P]2N_3NAD^+), we have detected nucleotide binding sites on rhIL-2. The specificity of these interactions indicates that nucleotide binding may play a role in the biological activity of IL-2.

MATERIALS AND METHODS

Materials. [γ-^{32}P]8N_3ATP (specific activity, 11–28 mCi/μmol; 1 mCi = 37 MBq) was prepared and purified as previously reported (20). The purity of [γ-^{32}P]8N_3ATP was determined by HPLC and TLC analysis (21). Following purification by DEAE-cellulose chromatography and HPLC, [γ-^{32}P]8N_3ATP was shown to be free of contaminating ATP, 8-bromo-ATP, 8-amino-ATP, or nucleoside 3′(2′)-phosphates. The synthesis of [α-^{32}P]2N_3NAD^+ (specific activity, 4–6 mCi/μmol) will be described elsewhere (H.K. and B.H., unpublished work). rhIL-2 either was from Cetus and supplied by the National Cancer Institute or was a gift from Hoffman–LaRoche. Protein molecular weight standards were from Bio-Rad. All other reagents were analytical grade and were from Sigma. Reaction buffer A consisted of 10 mM Tris (pH 7.1), 10 mM $MgCl_2$, and 5 mM KCl.

Photolabeling of rhIL-2. For saturation studies, samples containing 1–2 μg of rhIL-2 in 60 μl of buffer A were incubated at room temperature in Eppendorf tubes with either [α-^{32}P]2N_3NAD^+ or [γ-^{32}P]8N_3ATP for 15 sec prior to a 45-sec irradiation with a hand-held 254-nm UV lamp (intensity, 6200 $\mu W/cm^2$). After 45 sec of photolysis, the reaction was quenched by addition of a protein-solubilizing mixture consisting of 10% SDS, 3.6 M urea, 162 mM dithiothreitol, pyronin Y (tracking dye), and 20 mM Tris (pH 8.0). Additions to the basic reaction mixture described above were made as indi-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: 8N_3ATP, 8-azidoadenosine 5′-triphosphate; 8N_3GTP, 8-azidoguanosine 5′-triphosphate; 2N_3NAD^+; nicotinamide 2-azidoadenine dinucleotide; IL-2, interleukin 2; rhIL-2, recombinant human IL-2.

cated in the figure legends. Results reported in this paper have been reproduced a minimum of three to six times.

For protection studies, rhIL-2 in buffer A was first incubated 60 sec at room temperature with the competitor. At 60 sec the appropriate probe (10 or 20 μM) was added to the reaction mixture and at 75 sec the samples were then irradiated as above for 45 sec and solubilized in the protein-solubilizing mixture.

SDS/Polyacrylamide Gel Electrophoresis. After solubilization, samples were subjected to electrophoresis in a 10% separating gel with a 4% stacking gel according to the method of Laemmli (22). The gel was run at 35-mA constant current and stained for 1 hr with Coomassie brilliant blue R. Following destaining for 12 hr, the gel was dried on a slab gel dryer and subjected to autoradiography for 24–48 hr. Determination of ^{32}P incorporation was accomplished by excision of the labeled bands from the dried gel followed by scintillation counting in a Packard Minaxi scintillation counter (counting efficiency, 99% for ^{32}P).

RESULTS AND DISCUSSION

Many biological response modifiers such as IL-2 have molecular weights that are equal to or greater than those of many intracellular enzymes or toxins known to have specific catalytic properties involving nucleotides. Toxins are known to be internalized with the capability to express catalytic activity. IL-2 and similar polypeptide factors are known to bind to specific receptors and are subsequently internalized (15, 16) and degraded. However, it has not been determined whether internalization is required for the expression of the biological activity of IL-2 or other similar factors. Recently, major emphasis has been placed on studies of the IL-2 receptor(s) since they are thought to be the most likely candidates for transferring signal to the intracellular compartment. We initially developed a project on the effects of rhIL-2 on nucleotide-binding proteins in lymphokine-responsive cells. However, we were immediately concerned about the high molecular weights of IL-2 and related biological response modifiers as well as other polypeptide hormones. To us they appeared to be much higher than needed for activating a receptor through binding and they had the size capable of carrying catalytic activity. Therefore, based on the size of IL-2, its internalization, and its reported ability to stimulate intracellular phosphorylation (23), we set up studies to determine whether IL-2 was a nucleotide-binding protein. Three major events that are known to regulate intracellular biochemical pathways are (*i*) ATP-dependent phosphorylation, (*ii*) GTP binding to regulatory proteins, and (*iii*) NAD^+-dependent ADP-ribosylation. It was decided to test for these types of nucleotide binding sites. The 8-azido analogs of ATP and GTP have proven to be very effective photoprobes for a number of ATP- and GTP-binding proteins (23–28). Recent work with [α-^{32}P]$2N_3NAD^+$ photolabeling of glutamate and lactate dehydrogenases and isolation of their active-site peptides has demonstrated that this coenzyme analog will prove extremely useful for studying NAD^+-binding proteins (H.K. and B.H., unpublished work). Therefore, the following studies were done to determine the nature of any nucleotide binding site(s) that might exist on IL-2.

The specificity of azidonucleotide interaction with rhIL-2 was demonstrated by saturation effects as well as by competition for the binding site by the parent nucleotide at physiologically relevant concentrations. Saturable binding of [γ-^{32}P]$8N_3$ATP to rhIL-2 was accomplished by photolysis of reaction mixtures containing rhIL-2 (2 μg) in buffer A and various concentrations of the photoaffinity probe [γ-^{32}P]$8N_3$ATP (Fig. 1). Saturation of [γ-^{32}P]$8N_3$ATP photoinsertion into rhIL-2 occurred at 50 μM, satisfying the first criterion for specificity of nucleotide interaction. Under saturating conditions about 10 ± 2% of the IL-2 molecules were photolabeled in a similar experiment.

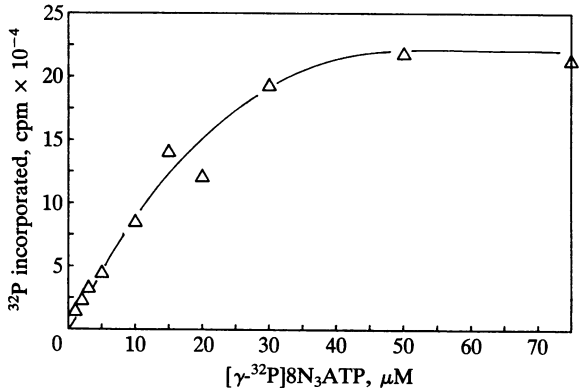

FIG. 1. Saturation of [γ-^{32}P]$8N_3$ATP photoincorporation into rhIL-2. One microgram of rhIL-2 in 60 μl of buffer A and the indicated concentration of photoaffinity probe were photolyzed and subjected to SDS/polyacrylamide gel electrophoresis. ^{32}P incorporation was detected by autoradiography and quantified by cutting out the appropriate band and determining radioactivity by liquid scintillation counting.

ATP effects on [γ-^{32}P]$8N_3$ATP photoinsertion into rhIL-2 were determined by using various concentrations of ATP to compete against binding and subsequent photolabeling of rhIL-2 by 10 μM [γ-^{32}P]$8N_3$ATP. Half-maximal prevention of [γ-^{32}P]$8N_3$ATP photoinsertion into rhIL-2 was obtained with 22 μM ATP (Fig. 2). The demonstration of saturation effects of [γ-^{32}P]$8N_3$ATP photoinsertion into rhIL-2 and the competition by ATP for photoinsertion into rhIL-2 at physiologically relevant concentrations indicate that the interaction of [γ-^{32}P]$8N_3$ATP with rhIL-2 is specific. Additionally, 100 μM AMP or ADP did not display nearly as great a protective effect as 100 μM ATP (data not shown).

Incubation of rhIL-2 with [γ-^{32}P]$8N_3$ATP in the reaction mixture in the absence of photolysis under identical conditions as used for photolyzed samples did not result in detectable ^{32}P incorporation into rhIL-2. This confirms that phosphorylation of rhIL-2 was not responsible for the observed radiolabeling.

The photoaffinity probe [α-^{32}P]$2N_3NAD^+$ was also tested for specific binding to rhIL-2. Saturation experiments were carried out in buffer A containing various concentrations of [α-^{32}P]$2N_3NAD^+$. Saturation effects were observed at 180 μM [α-^{32}P]$2N_3NAD^+$ (Fig. 3). Competition by NAD^+ for [α-^{32}P]$2N_3NAD^+$ (10 μM) binding and photoinsertion into

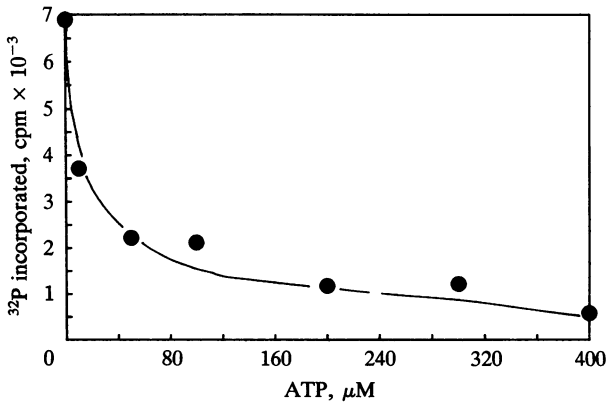

FIG. 2. Prevention of [γ-^{32}P]$8N_3$ATP photoinsertion into rhIL-2 by ATP. ATP was added to give the concentrations indicated in 60 μl of buffer A containing 1 μg of rhIL-2. After 1 min, [γ-^{32}P]$8N_3$ATP was added to give 10 μM, mixed 15 sec, and then photolyzed for 45 sec. ^{32}P incorporation was determined as in Fig. 1.

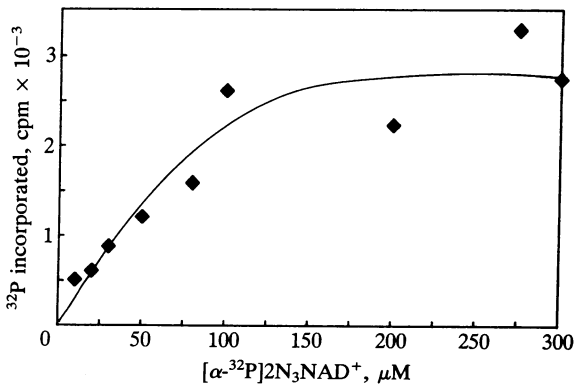

FIG. 3. Saturation of [α-^{32}P]2N$_3$NAD$^+$ photoincorporation into rhIL-2. One microgram of rhIL-2 was incubated 15 sec with the indicated concentrations of [α-^{32}P]2N$_3$NAD$^+$ and photolyzed. ^{32}P incorporation was determined as in Fig. 1.

rhIL-2 yielded half-maximal decrease of [α-^{32}P]2N$_3$NAD$^+$ photoinsertion at 10 μM NAD$^+$ (Fig. 4). Photoinsertion of [α-^{32}P]2N$_3$NAD$^+$ into rhIL-2 was also modified by divalent metal ions, as 10 mM EDTA decreased photoincorporation (see Table 1). Incubation of rhIL-2 with 10 μM [α-^{32}P]2N$_3$NAD$^+$ in the reaction mixture without photolysis did not result in detectable ^{32}P incorporation into rhIL-2, indicating a lack of an auto-ADP-ribosyltransferase activity under these conditions.

Effects of divalent metal ions on the photoinsertion of [γ-^{32}P]8N$_3$ATP and [α-^{32}P]2N$_3$NAD$^+$ into rhIL-2 are shown in Table 1. EDTA (10 mM) decreases [γ-^{32}P]8N$_3$ATP photoinsertion by >90%, whereas 10 μM $ZnCl_2$ enhances the photoinsertion by 80%. Mg^{2+} is required for [γ-^{32}P]8N$_3$ATP photoinsertion and $MnCl_2$ cannot substitute, whereas $CaCl_2$ seems to work slightly better. However, Ca^{2+} is not required, since 10 mM EGTA does not have the effect that EDTA does. The differential effects of Zn^{2+}, Ca^{2+}, and EGTA on [γ-^{32}P]8N$_3$ATP and [α-^{32}P]2N$_3$NAD$^+$ photoinsertion indicate that these two nucleotides differ in their metal-ion requirements for binding to IL-2. Also, experiments using NAD$^+$ and ATP to protect against the photoinsertion of both photoprobes indicate that the two sites are at different locations. In these experiments 50 μM NAD$^+$ prevented [α-^{32}P]2N$_3$NAD$^+$ photoinsertion by 90% and [γ-^{32}P]8N$_3$ATP photoinsertion by only 35%, whereas 50 μM ATP gave roughly the opposite effect. Also, GTP was not as effective as ATP at preventing [γ-^{32}P]8N$_3$ATP photoinsertion. Preliminary studies with [γ-^{32}P]8N$_3$GTP show that this analog also interacts with IL-2, probably at the same site as 8N$_3$ATP but with somewhat less affinity.

Table 1. Effects of divalent cations and nucleotide competitors on photoinsertion into rhIL-2

Addition	^{32}P incorporation, %	
	[γ-^{32}P]8N$_3$ATP	[α-^{32}P]2N$_3$NAD$^+$
Metal ions/chelators*		
10 mM $MgCl_2$	100	100
10 mM $MgCl_2$	10	15
10 mM $CaCl_2$	125	75
10 mM EDTA	9	35
10 mM EGTA	120	55
10 μM $ZnCl_2$	180	38
Competitors†		
5–200 μM Ap$_4$A	100	—
50 μM GTP	80	—
50 μM ATP	31	61
50 μM NAD$^+$	65	10

Photoinsertion obtained with either 10 μM [γ-^{32}P]8N$_3$ATP or 10 μM [α-^{32}P]2N$_3$NAD$^+$ in the presence of 10 mM $MgCl_2$ was taken as 100%.

*$ZnCl_2$ effects were determined in the presence of 10 mM $MgCl_2$; photoinsertion effects with other divalent metal ions were measured without addition of $MgCl_2$. Effects of the chelators EDTA and EGTA were measured without addition of $MgCl_2$.

†Competitors (at the given concentrations) were preincubated for 1 min at 23°C with 1 μg of rhIL-2 in reaction mixtures containing 10 mM $MgCl_2$. The appropriate photoprobe was then added to give a final concentration of 10 μM, and 15 sec later the samples were irradiated with UV light for 45 sec. Protein-solubilizing mixture was added immediately following light exposure and the samples were subjected to SDS/polyacrylamide gel electrophoresis. Ap$_4$A, adenosine(5')tetraphospho(5')adenosine.

A study utilizing 3-aminobenzamide as a competitor to prevent [α-^{32}P]2N$_3$NAD$^+$ photolabeling of rhIL-2 was undertaken because 3-aminobenzamide has been reported to inhibit some NAD$^+$-binding enzymes including, poly(ADP-ribose) polymerase and some mono(ADP-ribosyl)transferases (29). The concentration of 3-aminobenzamide required for 50% inhibition of enzymatic activity (IC_{50}) was reported to be 5.4 μM for poly(ADP-ribose) polymerase (*in vitro* and *in vivo*) and 3 mM for mono(ADP-ribosyl)transferase. In our study, half-maximal inhibition of [α-^{32}P]2N$_3$NAD$^+$ (10 μM) photoinsertion into rhIL-2 was achieved with ≈5 μM 3-aminobenzamide (Fig. 5). However, about 35% of the photoinsertion was insensitive to the inhibitor, which may

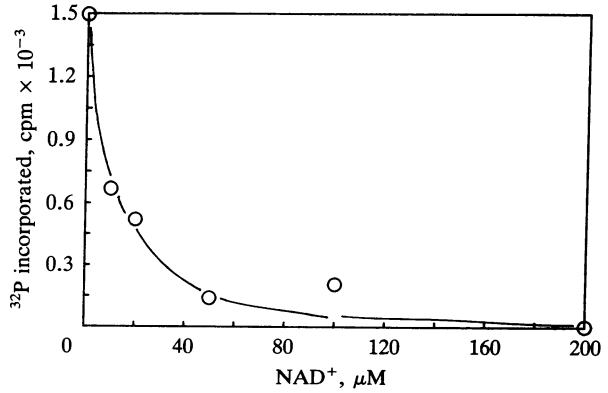

FIG. 4. Prevention of [α-^{32}P]2N$_3$NAD$^+$ photoinsertion into rhIL-2 by NAD$^+$. NAD$^+$ as indicated was added to 60 μl of buffer A containing 1 μg of rhIL-2 and allowed to incubate 1 min at 23°C prior to the addition of 10 μM [α-^{32}P]2N$_3$NAD$^+$. Fifteen seconds after probe addition, the sample was photolyzed and ^{32}P incorporation was determined as in Fig. 1.

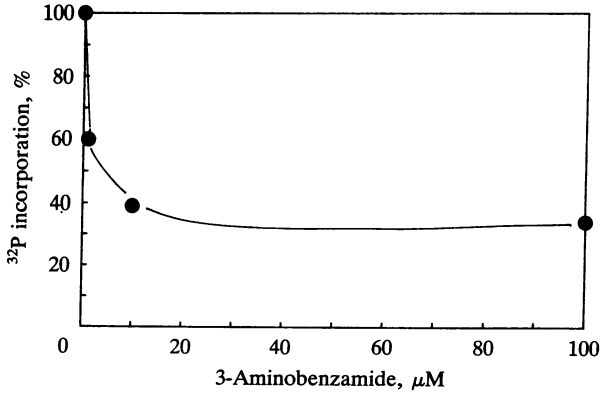

FIG. 5. Prevention of [α-^{32}P]2N$_3$NAD$^+$ photoincorporation into rhIL-2 by 3-aminobenzamide. Various amounts of 3-aminobenzamide were added to 60 μl of buffer A containing 1 μg of rhIL-2. Samples were incubated 1 min at 23°C prior to the addition of 10 μM [α-^{32}P]2N$_3$NAD$^+$. Fifteen seconds after probe addition, the sample was photolyzed and ^{32}P incorporation was determined as in Fig. 1.

represent the extent of NAD^+ crossbinding to the ATP site under these conditions as indicated by the data in Table 1.

An additional control experiment was performed for both [γ-^{32}P]8N_3ATP and [α-^{32}P]2N_3NAD^+ labeling studies of rhIL-2. Preirradiation of either photoprobe followed by immediate addition of protein (rhIL-2) generated no detectable incorporation of radioactivity into rhIL-2, indicating that the reactive species generated by photolysis is not long-lived. This result eliminates the possibility of pseudoaffinity labeling and is consistent with site-specific labeling of rhIL-2 by both azidonucleotides.

Additional evidence for the specificity and relevance of the binding of ATP by IL-2 comes from studies showing that rhIL-2 slowly autophosphorylates by an intramolecular mechanism. Incubation of 2 μg of rhIL-2 for 10 min at 23°C with 50 μM [γ-^{32}P]8N_3ATP (specific activity, 8.3 mCi/μmol) in the absence of activating light resulted in the incorporation of >40,000 cpm into the IL-2 as determined by scintillation counting of the protein band excised from an SDS/polyacrylamide gel. Instability of the autophosphorylated species to SDS/polyacrylamide gel electrophoresis made calculation of the percent phosphorylation a minimum, if not meaningless, number under these conditions. Dilution experiments in which the nucleotide concentration was kept constant while the 2 μg of IL-2 was diluted 2-, 3-, 4-, and 5-fold did not decrease the observed autophosphorylation rate, indicating an intramolecular mechanism. This type of experiment was done as previously reported for nucleoside-diphosphate kinase (30). These experiments have been repeated five times using rhIL-2 from two separate sources and nonphotoactive nucleotide. This phosphorylated species appears to be relatively unstable at all pH values but is most stable near neutrality and much less stable at both high (pH 11) and low (pH 1.0) pH values.

These results support the existence of specific binding sites for ATP and NAD^+ on rhIL-2 that would be occupied by nucleotide under intracellular conditions (i.e., millimolar levels for ATP and about 300 μM for NAD^+). Approximate dissociation constants obtained from the competition studies presented here were 22 μM for ATP and 10 μM for NAD^+, well within the physiologically relevant range. The precise mechanisms by which these nucleotide interactions with IL-2 may govern or regulate the biological activity of IL-2 are not understood but are suggestive of a possible intracellular signal-transduction mechanism that may *directly* involve IL-2. A recent report by Tigges *et al.* (31) indicates that the IL-2 signal mechanism does not involve any known second-messenger system. Our data indicate that IL-2 has the potential of being either a protein kinase or an ADP-ribosylating species, although neither of these activities has been confirmed with regard to any intracellular protein substrate. However, it seems quite unlikely that a nucleotide binding site of such high affinity would exist on IL-2 without being involved in eliciting at least some of the pleiotropic effects attributed to this protein. Additional support for the involvement of nucleotides in the mechanism of action of several glycoprotein hormones and other biological response modifiers comes from similar studies in our laboratory which show that tumor necrosis factor, interferon 1α, granulocyte/macrophage-colony-stimulating factor, interleukin 1, glucagon, and other similar proteins all contain sites that interact with specific nucleotide photoaffinity probes (unpublished results). They do not all have the same binding characteristics. For example, tumor necrosis factor demonstrates higher affinity for NAD^+ than for ATP, whereas the converse is seen with IL-2 (S.C. and B.H., unpublished work). Glucagon, which is much smaller, displays a high specificity for GTP (H.L. and B.H., unpublished work). These observations lead us to propose that many, if not all, polypeptide hormones or biological response modifiers require internalization and interaction with specific nucleotides or other intracellular moieties to elicit at least some of their effects. This hypothesis does not exclude the binding receptor from also being directly involved in the signal-transduction mechanism. The duality of receptor activation and internalization of a catalytically active protein may explain some of the pleiotypic effects of biological response modifiers.

This work was supported by National Institutes of Health Grant GM35766 to B.H.

1. Morgan, D. A., Ruscetti, R. W. & Gallo, R. C. (1976) *Science* **193,** 1007–1008.
2. Robb, R. J. (1985) *Behring Inst. Mitt.* **77,** 56–67.
3. Henney, C. S., Kruibayashi, K., Kern, D. E. & Gillis, S. (1983) *J. Immunol.* **130,** 222–227.
4. Flomenberg, N., Welte, K., Mertelsman, R., O'Reilly, R. & Dupont, B. (1983) *J. Immunol.* **130,** 2635–2643.
5. Domzig, W., Stadler, B. M. & Herberman, R. B. (1983) *J. Immunol.* **130,** 1970–1973.
6. Ezard, R., Corthesy, P., Nabholz, M., Lowenthal, J., Zaech, P., Plaetinck, G. & MacDonald, H. (1985) *J. Immunol.* **134,** 1644–1652.
7. Grimm, E. A., Ramsey, K. M., Mazumder, A., Wilson, D. J., Djeu, J. Y. & Rosenberg, S. A. (1983) *J. Exp. Med.* **157,** 884–897.
8. Merluzzi, V. J., Savage, D. M., Mertelsman, R. & Welte, K. (1984) *Cell Immunol.* **84,** 74–84.
9. Farrar, J., Benjamin, W., Hilfiker, M., Howard, M., Farrar, W. & Fuller-Farrar, J. (1982) *Immunol. Rev.* **63,** 129–166.
10. Robb, R. J., Greene, W. C. & Rusk, C. M. (1984) *J. Exp. Med.* **160,** 1126–1146.
11. Robb, R. J. & Greene, W. C. (1983) *J. Exp. Med.* **158,** 1332–1337.
12. Fuji, M., Sugamura, K., Sano, K., Nakai, M., Sugita, K. & Hinuma, Y. (1986) *J. Exp. Med.* **163,** 550–562.
13. Weissman, A., Harford, J., Svetlik, P., Leonard, W., Depper, J., Waldmann, T., Greene, W. & Klausner, R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1463–1466.
14. Evans, S. W., Beckner, S. K. & Farrar, W. L. (1987) *Nature (London)* **325,** 166–168.
15. Robb, R. J., Kunty, R. M. & Chowdry, V. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5990–5994.
16. Taniguchi, T., Matsi, H., Fugita, T., Takaoka, C., Kashima, N., Yoshimoto, R. & Jamuro, J. (1983) *Nature (London)* **302,** 305–310.
17. Devos, R., Plaetinck, G., Cheroutre, H., Simons, G., Degrave, W., Tavernier, J., Remaut, E. & Fiers, W. (1983) *Nucleic Acids Res.* **11,** 4307–4323.
18. Rosenberg, S. A., Grimm, E. A., McGrogan, M., Doyle, M., Kawasaki, E., Koths, K. & Mark, D. F. (1984) *Science* **223,** 1412–1415.
19. Clark, S. C., Arya, S. K., Wong-Staal, F., Matsumono-Kobayashi, M., Kay, R. M., Kaufman, R. J., Brown, E. L., Shoemaker, C., Copeland, T., Oroszlan, S., Smith, K., Sarngadharan, M. G., Lindner, S. G. & Gallo, R. C. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2543–2547.
20. Haley, B. E. (1977) *Methods Enzymol.* **46,** 339–346.
21. Czarnecki, J. J., Abbot, M. S. & Selman, B. R. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7744–7748.
22. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
23. Gaulton, G. N. & Eardley, D. D. (1986) *J. Immunol.* **136,** 2470–2477.
24. Potter, R. L. & Haley, B. E. (1983) *Methods Enzymol.* **91,** 613–633.
25. Haley, B. E. (1975) *Biochemistry* **14,** 3852–3857.
26. Geahlen, R. & Haley, B. E. (1979) *J. Biol. Chem.* **254,** 11982–11987.
27. Suhadolnik, R. L., Li, Shi Wu, Sobol, R. W., Jr. & Haley, B. E. (1988) *Biochemistry* **27,** 8846–8851.
28. Julin, D. A. & Lehman, I. R. (1987) *J. Biol. Chem.* **262,** 9044–9051.
29. Rankin, P. W., Jacobson, E. L., Benjamin, R. C., Moss, J. & Jacobson, M. K. (1989) *J. Biol. Chem.* **264,** 4312–4317.
30. Francis, B., Overmeyer, J., Marshall, E. & Haley, B. (1989) *Mol. Carcinogen.* **2,** 168–178.
31. Tigges, M. A., Casey, L. S. & Koshland, M. E. (1989) *Science* **243,** 781–786.