Adenine Binding Sites of the Lectin from Lima Beans (*Phaseolus lunatus*)

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David D. Roberts§ and Irwin J. Goldstein§

From the Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48109

A single high-affinity binding site for adenine and related compounds was identified in the Lima bean lectin (LBL) component III tetramer. This site is identical with the high affinity site for 2,6-toluidinynaphthalenesulfonate described previously (Roberts, D. D., and Goldstein, I. J. (1982) *J. Biol. Chem.* 257, 11274–11277). [14C]Adenine was bound with high affinity ($K_d = 1.2 \pm 0.1 \times 10^{-5}$ M, $T = 25^\circ$C) and a high degree of specificity in that hypoxanthine and guanine were very poor ligands for this site. Specificity was also observed for free purine bases relative to nucleosides or nucleotides. A number of N$^0$ derivatives of adenine with cytokinin activity were found to bind to LBL, with relative affinities decreasing in the order: N$^0$-benzyladenine > kinetin > zeatin > N$^0$-1,8-anilinonaphthalenesulfonate. Evidence was also obtained for heterotropic interaction between the adenine binding site and a second class of hydrophobic sites present on each subunit of LBL. Binding of adenine and N$^0$-benzyladenine to LBL was found to produce a 2.3- and 3.8-fold increase, respectively, in the affinity of the lectin subunit hydrophobic sites for 1,8-anilinonaphthalenesulfonate. 1,8-Anilinonaphthalenesulfonate, in turn, enhanced the affinity of LBL for adenine, demonstrating that binding of ligands to the two classes of hydrophobic sites is thermodynamically linked. Equilibrium dialysis also revealed high affinity binding sites for [14C]adenine on the lectins from *Dolichos biflorus*, *Phaseolus vulgaris*, and soybean (*Glycine max*).

The lectin isolated from Lima beans (*Phaseolus lunatus*) specifically recognizes glycoconjugates containing terminal nonreducing α-3-GalNAc residues (1, 2). The lectin occurs and may be isolated in several aggregation states (3–5). The predominant form, component III, is a tetramer of 31,000-dalton subunits which is divalent for GalNAc (6). In addition to specific binding sites for carbohydrate, the lectin was found to possess two classes of sites which bind nonpolar ligands (7). Tetrameric component III has a single high affinity binding site for 2,6-toluidinynaphthalenesulfonic acid and four additional sites (one on each subunit) which bind TNS$^+$ or 1,8-anilinonaphthalenesulfonic acid. These ligands were also shown to interact with the lectin from jack beans, concanavalin A (8), and with ricin (9). We recently demonstrated that a number of additional lectins also bind ANS and TNS and suggested that ANS binding sites are conserved in a majority of legume lectins (10).

The function of hydrophobic ligand binding in legume lectins, as well as that of carbohydrate binding is unknown. ANS and TNS bind to a number of proteins at cofactor or hormone binding sites (see Ref. 11 for review). Edelman and Wang examined the interaction of the phytohormone indoleacetic acid with concanavalin A and found weak binding with $K_d = 7 \times 10^6$ M$^{-1}$ (12). A class of N$^0$ substituted adenine derivatives, the cytokinins (4), are also present in plants and serve an important role as phytohormones (13, 14). These compounds are similar in structure to one of the fluorescent ligands shown previously to bind to the Lima bean lectin, N-phenyl-1-naphthylamine (2). This prompted us to characterize the interactions of LBL with cytokinins.

In the present communication we report the binding properties of LBL for possible physiological hydrophobic ligands including the cytokinins and several common cofactors. The results of these studies demonstrate the presence of a highly specific site on LBL for adenine derivatives. Remarkably, occupation of this site induced changes in the affinity of the subunit hydrophobic sites for ANS.

**MATERIALS AND METHODS**

LBL was prepared as described previously by affinity chromatography on Synsorb A (6, 15). Component III was prepared by diethreitol reduction of the purified isolectins (15). [14C]Adenine was purchased from ICN. Adenine sulfate and ANS were obtained from Eastman Kodak. ANS was recrystallized from water as the magnesium salt. Most other purines, cofactors, and cytokinins were obtained from Sigma. *Dolichos biflorus* lectin was provided by Dr. M. E. Etzler (University of California, Davis). Soybean agglutinin and *Phaseolus vulgaris* erythroglobulin were provided by Dr. E. Chu from E Y Laboratories (San Mateo, CA).

UV spectra were recorded with a 1-nm bandwidth on a Cary 219 spectrophotometer. Difference spectra were done using 0.88-cm split compartment cells and 1 ml each of lectin and ligand in PBS.

Equilibrium dialysis was conducted in microdialysis cells (model 346, Technilab Instruments, Inc., Pequannock, NJ) using 0.3-ml volume of lectin and ligands in PBS. Following equilibration for 24 h on a shaking bath at 25 °C, the concentration of [14C]adenine in each chamber was determined by scintillation counting in ACS (Amer sham, Arlington Heights, IL).

Fluorescence titrations were done as described previously (7) using...
an SLM 8000 photon counting spectrofluorometer in ratio mode, thermostatted at 25 °C. An excitation band width of 2 nm and an emission band width of 4 nm were used for all measurements.

Dissociation constants were determined by regression analysis of binding data and are presented with errors determined as the standard error of the regression coefficients. Lima bean lectin concentrations were determined by absorbance at 280 nm using \( E_{\text{1} \, \text{cm} \, \text{mM}} = 12.3 \) (3).

**RESULTS**

Initially, UV difference spectroscopy was used to examine interaction between several enzyme cofactors and LBL. No perturbations were observed using FAD or FMN. Oxidized and reduced pyridine nucleotides and nucleotide phosphates gave weak difference spectra at 260 nm. In all cases, however, the affinity was too low to determine a \( K_d \) by UV difference titration. Similar difference spectra were obtained using 5'-AMP and adenosine. The UV spectrum of \( {N^\circ} \)-benzyladenine, in contrast, was significantly altered in the presence of LBL (Fig. 1). UV difference titration using 2.75 μM LBL (assuming \( M_r = 124,000 \)) with 15 to 100 μM BAP gave \( K_d = 37 \pm 6 \) μM.

Nonfluorescent ligands were next examined in a competition assay based on their ability to displace the fluorescent ligand ANS from the lectin. Inasmuch as a large enhancement of ANS fluorescence was observed on binding to LBL, binding of a competing ligand was expected to decrease the fluorescence emission of a lectin/ANS mixture. ANS was chosen since it was shown to bind in a relatively specific manner to the subunit hydrophobic sites, whereas TNS bound to both classes of sites (7). A number of ligands were surveyed for binding using this competition assay. Only weak inhibition was seen with most ligands including the cofactors which gave weak difference spectra with LBL. In contrast to the decrease in ANS fluorescence observed with the above ligands, an unexpectedly large increase in ANS fluorescence was seen when BAP was added to LBL containing 100 μM ANS (Fig. 2). Controls lacking lectin indicated no direct effect of BAP on ANS fluorescence. Titration of an ANS/LBL mixture with BAP demonstrated that the enhancement was a saturable phenomenon with half-maximal enhancement seen at 28 μM BAP (inset, Fig. 2). This finding suggested that binding of BAP to a site distinct from the ANS sites (e.g. the high affinity TNS site) induced either an increase in the affinity for ANS or an increase in the quantum yield of ANS bound to the lectin. To distinguish between these two possibilities, ANS titrations were done at several fixed concentrations of BAP (Fig. 3). The binding data are presented in a linearized form according to Equation 1, where \( \Delta F \) is the net enhancement of ANS fluorescence, \( \Delta F_0 \) is the enhancement at saturating ANS, and \( K \) is the apparent dissociation constant for ANS.

\[
\frac{\Delta F}{[\text{ANS}]} = \frac{\Delta F_0}{K} = \frac{\Delta F}{K}
\]

**Fig. 1.** UV difference spectrum of BAP and lima bean lectin. After mixing, the composition was 0.36 mg/ml of lima bean lectin and 50 μM BAP in PBS, pH 6.8.

**Fig. 2.** Effect of BAP on ANS fluorescence in the presence of lima bean lectin. Fluorescence emission spectra for ANS (100 μM) were determined at 25 °C with excitation at 550 nm. The recorded spectra for ANS contain the following: no addition or 146 μM BAP (a), 0.57 mg/ml of LBL (b), 0.57 mg/ml of LBL and 20 μM BAP (c), 0.57 mg/ml of LBL and 49 μM BAP (d), 0.57 mg/ml of LBL and 146 μM BAP (e). Inset, net fluorescence enhancement of 100 μM ANS was determined in the presence of 0.41 mg/ml of LBL and 0–300 μM BAP; excitation was at 240 nm and emission at 480 nm. Corrections were made for dilution and fluorescence of free ANS.

**Fig. 3.** Effect of BAP on ANS binding to lima bean lectin. Binding of ANS to LBL was determined using specific enhancement of ligand fluorescence corrected for fluorescence of free ligand and dilution. Binding data are presented as Scatchard plots according to Glaudemans and Jolley (25). Lectin (0.4 mg/ml) was titrated with ANS (0.4 mg/ml) in PBS pH 6.8 (○) or PBS containing 10 μM BAP (●), 20 μM BAP (●), 50 μM BAP (○), and 200 μM BAP (▲); excitation was at 420 nm and emission at 480 nm.

If BAP alters the quantum yield of bound ANS, which is proportional to \( \Delta F_0 \), the x intercept would change, whereas if BAP alters the affinity of LBL for ANS for the quantum yield is unchanged then the slope will be altered but the x intercept will be constant. These plots showed the quantum yield of bound ANS to be independent of BAP concentration, whereas the affinity for ANS increased with increasing BAP.

If the BAP-induced enhancement of ANS binding was a consequence of BAP binding to the high affinity TNS site, BAP would be expected to partially displace TNS. Approximately 25% inhibition of TNS fluorescence enhancement was seen upon addition of 200 μM BAP to LBL containing 1.5 μM TNS. Titrations of LBL with TNS in the presence and absence of BAP (200 μM) indicated that BAP selectively inhibited the high affinity binding of TNS to LBL without blocking TNS binding to the low affinity subunit sites (Fig. 4). Due to the heterogeneity of TNS binding and the possibility that BAP may allosterically alter low affinity TNS binding as well as ANS binding, the effect of BAP on high affinity TNS binding could not be quantified. However, the observed partial inhibition is consistent with selective inhibition of high affinity TNS binding due to competition by
BAP for this site. The stoichiometry of high affinity TNS binding has been determined previously as 1 site/tetramer (7). Thus, the stoichiometry of BAP binding is also predicted to be 1 site/tetramer.

The structural similarity between BAP and ANS suggests that BAP should also bind to the subunit hydrophobic sites. At concentrations of BAP greater than 500 μM some inhibition of ANS fluorescence enhancement was observed. Similarly, high BAP concentrations decreased TNS fluorescence enhancement beyond that expected for inhibition of high affinity TNS binding. Thus BAP may also bind to the subunit hydrophobic sites, albeit with lower affinity.

Enhancement of ANS binding was also observed when adenine and a number of related ligands were introduced into a solution of LBL containing 100 μM ANS. This technique was used to screen a number of adenine analogs for binding to LBL. Concentrations of ligand producing half-maximal “activation” of ANS binding in this assay are listed in Table I.

The above assay is only an indirect measure of ligand binding. In addition, the assumption cannot be made that the relationship between ANS binding and adenine derivative binding is linear. Thus, the activation constants are not necessarily equal to dissociation constants for the ligands tested. To directly measure affinity and stoichiometry of adenine analog binding, equilibrium dialysis was conducted using radiolabeled adenine. Equilibrium dialysis of LBL component III (1.0 mg/ml) was equilibrated with 4-80 μM [14C]adenine in PBS for 24 h at 25°C on a shaking bath. Adenine concentrations in each compartment were determined by counting duplicate 100-μl aliquots. Stoichiometry of binding (n) was calculated using n = 124,000 for the lectin tetramer.

![FIG. 4. Effect of BAP on TNS binding to lima bean lectin.](image)

**TABLE I**

<table>
<thead>
<tr>
<th>Ligand</th>
<th><strong>K&lt;sub&gt;activation&lt;/sub&gt;</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>1.8 × 10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
<tr>
<td>N&lt;sup&gt;6&lt;/sup&gt;-Benzyladenine</td>
<td>2.8 × 10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kinetin</td>
<td>3.9 × 10&lt;sup&gt;-4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zeatin</td>
<td>5.2 × 10&lt;sup&gt;-5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isopenenyladenine</td>
<td>8.2 × 10&lt;sup&gt;-5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dihydrozeatin</td>
<td>2.2 × 10&lt;sup&gt;-4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zeatin ribosite</td>
<td>~3 × 10&lt;sup&gt;-4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hyposaxanthine</td>
<td>~5 × 10&lt;sup&gt;-4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Guanine</td>
<td>~5 × 10&lt;sup&gt;-4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adenosine</td>
<td>&gt;10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
<tr>
<td>2′-Deoxyadenosine</td>
<td>&gt;10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
<tr>
<td>3′-5′-cAMP</td>
<td>&gt;10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>&gt;10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
<tr>
<td>5′-AMP</td>
<td>&gt;10&lt;sup&gt;-2&lt;/sup&gt;</td>
</tr>
<tr>
<td>NAD</td>
<td>&gt;10&lt;sup&gt;-8&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Concentrations of ligand giving half-maximal enhancement of ANS binding at 25°C.

<sup>b</sup> Weak inhibitors of ANS binding.

![FIG. 5. Binding of [14C]adenine to lima bean lectin by equilibrium dialysis.](image)

**TABLE II**

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Ligand</th>
<th><strong>K&lt;sub&gt;d&lt;/sub&gt;</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>LBL</td>
<td>Adenine</td>
<td>1.2 ± 0.1 × 10&lt;sup&gt;-5&lt;/sup&gt;</td>
</tr>
<tr>
<td>LBL</td>
<td>N&lt;sup&gt;6&lt;/sup&gt;-Benzyladenine</td>
<td>2.4 ± 0.1 × 10&lt;sup&gt;-5&lt;/sup&gt;</td>
</tr>
<tr>
<td>LBL</td>
<td>Zeatin</td>
<td>9.1 ± 0.9 × 10&lt;sup&gt;-5&lt;/sup&gt;</td>
</tr>
<tr>
<td>LBL</td>
<td>Hypoxanthine</td>
<td>1.2 ± 0.3 × 10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
<tr>
<td>D. biflorus</td>
<td>Adenine</td>
<td>2.0 × 10&lt;sup&gt;-4&lt;/sup&gt;</td>
</tr>
<tr>
<td>PHA-E&lt;sub&gt;a&lt;/sub&gt;</td>
<td>Adenine</td>
<td>8.6 × 10&lt;sup&gt;-4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soybean agglutinin</td>
<td>Adenine</td>
<td>1.3 × 10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> PHA-E<sub>a</sub>, P. vulgaris erythroagglutinin.

**Fig. 5. Binding of [14C]adenine to lima bean lectin by equilibrium dialysis.** Lima bean lectin component III (1.0 mg/ml) was equilibrated with 4-80 μM [14C]adenine in PBS for 24 h at 25°C on a shaking bath. Adenine concentrations in each compartment were determined by counting duplicate 100-μl aliquots. Stoichiometry of binding (n) was calculated using n = 124,000 for the lectin tetramer.

Using radiolabeled adenine. Equilibrium dialysis of LBL component III with [14C]adenine confirmed a stoichiometry of 1 site/tetramer (n = 1.23 assuming M<sub>r</sub> = 124,000) and gave K<sub>d</sub> = 1.2 ± 0.1 × 10<sup>-5</sup> M (Fig. 5). Identical K<sub>d</sub> and stoichiometry, on a tetramer basis, were observed using a natural mixture of lectin components I, II, and III.

Binding of adenine to several other legume lectins which have been shown to bind ANS and TNS (10) was also examined by equilibrium dialysis (Table II). High affinity binding sites for adenine were identified on soybean agglutinin, P. vulgaris erythroagglutinin, and D. biflorus lectin. Griffonia simplicifolia I-A<sub>1</sub>, isolectin (1.0 mg/ml) did not bind [14C] adenine on equilibrium dialysis, indicating that the interaction of adenine with the ANS or TNS sites of this lectin must be weak (K<sub>d</sub> > 10<sup>-4</sup> M).

[14C]Adenine was also used as a tracer to determine the dissociation constants for several additional ligands in a competitive binding assay (16). Equilibrium dialysis was performed with varying competing ligand concentrations and a constant concentration of [14C]adenine (5 μM). Bound competing ligand was determined from displacement of [14C] adenine. For two competing ligands B<sub>1</sub> and B<sub>2</sub> binding to a single site on lectin (P), the following equilibria exist.

\[ P + B_1 \rightleftharpoons K_1 P B_1 \]
\[ + \]
\[ B_2 \rightleftharpoons K_2 P B_2 \]

K<sub>1</sub> and K<sub>2</sub> represent dissociation constants for the respective ligands. Using the law of mass action, bound competing ligand (B<sub>i</sub>) can be expressed in terms of measured bound and free adenine (B<sub>j</sub>), where \( v_1 \) and \( v_2 \) represent the stoichiometry of bound B<sub>1</sub> and B<sub>2</sub>, respectively, and [P]<sub>0</sub> is the lectin concentration on one side of the cell.

\[ v_2 = 1 - v_1 \frac{[PB_1]K_2}{[P]_0[B_2]} \]
Free competing ligand $[B_2]_{	ext{free}}$ can then be determined using Equation 4 where $[B_2]_{	ext{total}}$ is given as $B_2$ added/total cell volume.

$$[B_2]_{\text{free}} = [B_2]_{\text{total}} - \frac{pB_2}{2}$$

Scatchard plots were constructed using these values (17). Dissociation constants for the competing ligands, determined by linear regression analysis, are listed in Table II.

Since binding of adenine derivatives to the high affinity TNS site increased the affinity of LBL for ANS, the binding of these two ligands appears to be linked. The binding of two ligands, ANS (A) and cytokinin (B) to LBL (P) can be written as a thermodynamic cycle.

$$P + 4A + B \rightleftharpoons PA_4 + B$$

$$K_{A,D} \rightleftharpoons PA + B + PB$$

$K_{A(D)}$ represent dissociation constants for the indicated ligands in the presence or absence of the second ligand. For simplicity, the dissociation constant for binding of ANS to one subunit site is assumed to be independent of occupancy of the other three subunit sites and equal to $K_A$. This assumption is supported by the lack of cooperativity in previous studies of ANS binding to LBL (7). For the above thermodynamic cycle the following relationship must be satisfied.

$$K_A K_c = K_{PB} K_{Pa}$$

Values for $K_A (2.6 \times 10^{-4} \text{ M})$ have been measured by fluorescence titration and equilibrium dialysis (7) and for $K_c (1.2 \times 10^{-5} \text{ M})$ by equilibrium dialysis. From titrations of ANS in the presence of BAP the affinity for ANS at saturating levels of BAP was estimated ($K_{PB} = 6.9 \pm 0.5 \times 10^{-5} \text{ M}$). Using these values in Equation 6, the affinity for BAP at saturating ANS can also be calculated ($K_c \approx 6 \times 10^{-8} \text{ M}$). The large enhancement of BAP affinity induced by ANS binding ($K_c$ versus $K_B$) is a consequence of the 4:1 stoichiometry of ANS binding.

We wanted to directly measure the ability of ANS to enhance the affinity of adenine in order to test the linkage relationship given in Equation 6. Adenine was chosen for this purpose since it was used as labeled ligand in equilibrium dialysis experiments. In experiments analogous to those presented in Fig. 3, ANS titrations were done at several fixed adenine concentrations, giving a dissociation constant for ANS in the presence of saturating adenine ($K_A = 1.1 \pm 0.1 \times 10^{-4} \text{ M}$). Using Equation 6, the expected dissociation constant for adenine in the presence of excess ANS was then determined ($K_c \approx 4 \times 10^{-7} \text{ M}$). Equilibrium dialysis with $[^{14}C]$ adenine at a saturating ANS concentration (2 mM) failed to show the expected increase in affinity for adenine. However, at subsaturating ANS levels the expected increase was observed. Using 94 $\mu$M ANS, LBL should be 26% saturated by ANS, with an average of 1 ANS bound/tetramer. If ANS and adenine binding are linked, the affinity of adenine should be increased 2.3-fold or $K_{PB} = 5.2 \times 10^{-6} \text{ M}^{-1}$. Equilibrium dialysis of adenine at 94 $\mu$M ANS gave $K_{PB} = 6.6 \times 10^{-6} \text{ M}^{-1}$ in reasonable agreement with the predicted value. Similar experiments at several ANS concentrations gave maximal stimulation of adenine binding at 300 $\mu$M ANS, whereas higher concentrations began to inhibit adenine binding. The decrease in adenine affinity at high ANS concentration may be due to weak binding of ANS to the adenine site, competing with adenine binding. Mixed allosteric activation and competitive inhibition have been observed in other allosteric proteins including aspartate transcarbamylase (18).

Control experiments were done to ensure that the deviation of adenine binding from that predicted by linkage could not be due to instability of adenine. If, in the presence of ANS, the lectin catalyzed conversion of adenine to a nonbinding form (e.g. hydrolysis to hypoxanthine), binding affinity would be lowered. Analysis by cellulose thin layer chromatography (19) of adenine from the above dialysis experiments did not reveal the presence of hypoxanthine or any radiolabeled compounds other than adenine.

**DISCUSSION**

A single high affinity site for adenine and cytokinins has been identified on the lima bean lectin tetramer. Three methods used to assess binding parameters for adenine analogs gave good agreement. Due to the complex interactions between ligands, direct binding measurement by equilibrium dialysis was the most reliable method for measuring affinities of adenine and its derivatives. This approach established the stoichiometry for adenine binding, 1 site/tetramer, and gave affinities for adenine, BAP, zeatin, and hypoxanthine. Determination of BAP binding by UV difference spectroscopy gave a somewhat higher $K_c$ than measured by dialysis. This could be due to the limited range of concentration used for difference spectroscopy or could result from contributions due to binding of BAP to the subunit sites. The $K_c$ for BAP from equilibrium dialysis represents only binding to the single high affinity site. The results of equilibrium dialysis also validate the ordering of potency determined using ANS fluorescence enhancement. In both methods the relative affinities observed were: adenine > BAP > zeatin > hypoxanthine.

The detailed structural requirements for binding of adenine analogs remains to be determined. A few general trends emerge from the relative activities of ligands tested in the ANS fluorescence assay. First, a strong preference for $N^6$-amines was observed. Binding of hypoxanthine, with an hydroxyl at the 6 position, was approximately 25-fold weaker than adenine by the fluorescence assay and 100-fold weaker by equilibrium dialysis. Second, there is a strong preference for free bases over nucleosides or nucleotides. This preference is also consistent with the low affinity of pyridine nucleotides. Substitution by alkyl and alkylaryl groups at the $N^6$-amine is tolerated. Both synthetic and naturally occurring cytokinins were bound. Adenine derivatives with unsaturated or aromatic side chains at $N^6$ were better ligands than derivatives with saturated side chains.

Linkage has been demonstrated between binding of adenine derivatives to the high affinity TNS site and binding of ANS to the subunit hydrophobic sites of LBL. At low levels of ANS, interactions between ANS and adenine binding can be quantitatively described by the thermodynamic cycle in Equation 5. The relative alteration and the affinity of LBL for ANS in the presence of adenine and BAP is also notable. Even though adenine binds to LBL with higher affinity than BAP, BAP produces a larger enhancement of ANS binding affinity than adenine. Using Equation 6 and ignoring cross-reaction of ANS with the adenine site, this observation led to the prediction that the affinity for BAP at saturating ANS ($K_c = 6 \times 10^{-6} \text{ M}$) is greater than the affinity for adenine under the same conditions ($K_c = 4 \times 10^{-7} \text{ M}$). In the absence of ANS, the affinity of LBL for adenine is twice that for BAP, $K_B$ (adenine) < $K_B$ (BAP). Thus in the presence of ANS, the specificity of the site is altered so that BAP is bound with higher affinity than adenine.

Hydrophobic ligand binding has been found to be widespread in legume lectins (10) and characterized in detail for LBL and concanavalin A using fluorescent ligands (7, 8). Whereas these interactions are interesting, they do not represent binding of naturally occurring ligands. Furthermore,
probe binding sites do not always correlate with known functional sites in proteins (11). In some cases they may be nonfunctional. The demonstration here of adenine and cytokinin binding to LBL identifies specificity for ligands known to be present in legumes. Thus, the high affinity TNS site may be of physiological importance. On the other hand, no ligands have been identified which bind with high affinity to the subunit hydrophobic sites. Linkage between ANS and adenine derivative binding is intriguing in that it provides strong evidence for alterations in the properties of LBL in response to naturally occurring ligands. The affinity of these ligands for LBL may also be regulated by naturally occurring structural analogs of ANS.

Cytokinin binding proteins have been isolated from several plant sources including wheat germ (20–22) and tobacco cells in culture (23, 24). Affinity constants of these proteins for cytokinins ranged from $10^7$ to $10^8$ M$^{-1}$. The function of these proteins in mediating cytokinin actions is unclear, although a receptor purified from wheat germ has been reported to bind to ribosomes (22). The affinity of purified lima bean lectin for cytokinins is lower than determined for some other receptors. The affinity may be much higher, however, in the presence of activators analogous to ANS. Lectins are present in legumes. Thus, the high affinity TNS site may be of physiological importance. On the other hand, no evidence for alterations in the properties of LBL in response to cytokinins is lower than determined for some other receptors. Whether legume lectins could have other functions in cytokinin-dependent processes remains to be determined.

Several important questions remain to be answered. Is binding of adenine derivatives a general property of legume lectins? Does this binding alter the properties of the other lectins as observed with LBL? High affinity adenine binding sites have been identified in lectins from soybean, *P. vulgaris*, and *D. biflorus*. Preliminary experiments with *D. biflorus* lectin indicate that BAP is also a good ligand for this lectin. In some, but not all of these lectins, binding of adenine or cytokinins is linked to changes in ANS binding. In contrast, *G. simplicifolia* I-A, isolectin did not bind adenine with high affinity. Thus considerable variation in binding affinity and perhaps in specificity for adenine analogs is expected for legume lectins.

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