Conformational and Allosteric Changes in Fructose 1,6-Bisphosphatase upon Photoaffinity Labeling with 2-Azidoadenosine Monophosphate*

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The photoaffinity analog 2-azido-AMP was found to be a potent allosteric inhibitor of pig kidney fructose 1,6-bisphosphatase. UV-induced covalent incorporation of 2-azido-[8-3H]AMP fully inactivated the enzyme at a level stoichiometric with its subunit composition (4 mol of analog/mol of tetramer). The photoincorporation and inactivation were prevented by the presence of AMP but not by the substrate, IMP, or adenosine. Enzyme fully modified with 2-azido-AMP was capable of binding fructose 1,6-bisphosphate but not AMP. The analog thus specifically modified the enzyme’s allosteric sites.

Titration of the native enzyme’s fast reacting cysteines with 5,5'-dithiobis-(2-nitrobenzoic acid) distinguished four conformational states of the enzyme: $E(\pm Mg^2+)$, $E(AMP)$, $E(AMP)Mg^2+$, $E(Fru-1,6-P_2)x$. AMP had a biphasic effect with a low affinity phase that could be mimicked by IMP, a competitive inhibitor of fructose 1,6-bisphosphate binding. Enzyme fully modified with 2-azido-AMP was isolated and observed to have a similar conformation and to undergo similar ligand-induced conformational changes as the native enzyme-AMP complex.

Random modification of 50% of the subunits with 2-azido-AMP shifted the AMP inhibition curve from sigmoidal to hyperbolic without changing the inhibition constant. AMP-induced cooperativity in the pig kidney fructose 1,6-bisphosphatase appears to be restricted to 2 subunits without alterations in AMP affinity.

Fructose 1,6-bisphosphatase (EC 3.1.3.11, d-fructose-1,6-bisphosphatase 1-phosphohydrolase) catalyzes one of the key regulatory steps of gluconeogenesis. The enzyme from most sources is a tetramer of identical subunits, each containing one site for the substrate fructose 1,6-bisphosphate and one site for the allosteric inhibitor AMP (for a review see Ref. 1). The enzyme is generally considered to be regulated in vivo via the allosteric interactions of AMP (2). Structural requirements for nucleotide binding are very specific for a 5'-monophosphate and the 6-NH$_2$ group of adenine; IMP and GMP are not allosteric inhibitors of the enzyme (2). Nuclear magnetic resonance studies of the rabbit liver enzyme indicate that the AMP is bound in an anti conformation (3). A study on the pig kidney and muscle fructose 1,6-bisphosphatases with the photoaffinity analog 8-N$_2$AMP$^1$ has been performed (4). Since 8-substituted adenine nucleotides are known to prefer a syn conformation (5), nucleotide conformational restrictions may explain the low binding affinity for the 8-azido analog (4). This study utilizes the photoaffinity analog 2-azido-AMP as a probe of the structure and function of the pig kidney fructose 1,6-bisphosphatase. The 2-azido substitution should allow the nucleotide to adopt an anti-conformation while retaining the required 6-NH$_2$ and 5'-monophosphate structures. Several reports using 2-azidoadenine nucleotides as photoaffinity probes have been published (6–8).

**MATERIALS AND METHODS**

Pig kidney fructose 1,6-bisphosphatase was purified as described (9) with an extra heating step at 62°C for 5 min (4). The purified enzyme had a neutral pH optimum and a specific activity of 26–35 units/mg of protein. SDS-gel electrophoresis (10) showed a major band of $M_r = 35,000$ and one small band of $M_r = 29,000$. The molecular weight was assumed to be 140,000.

Enzyme activity was measured at 30°C using coupled enzyme or phosphate release assays. The coupled enzyme assay followed the continuous production of NADPH in a 1-ml volume containing 50 mM HEPES-Na, pH 7.5, 0.1 mM EDTA, 150 mM KCl, 0.2 mM NADP$, 0.14$ mM Fru-1,6-P$_2$, 3.5 units/ml of phosphoglucose isomerase, and 0.7 unit/ml of glucose-6-phosphate dehydrogenase. The phosphate release assay medium was identical except for the omission of the auxiliary enzymes and NADP$^+$ and a decrease in the Fru-1,6-P$_2$ concentration to 70 μM. The inorganic phosphate produced after 5 min was determined by a modification of the method of Lanzetta et al. (11). The reaction was stopped by the addition of 2 ml of the color reagent (1.28% ammonium molybdate, 0.042% malachite green, 1.2 N HCl). After 5 min, 0.3 ml of the citrate-detergent solution (17% trisodium citrate, 0.5% Tween 20, 0.6 N HCl) was added and the samples were read at 660 nm. The assay was linear with time and with enzyme concentration. All enzyme samples were diluted at least 3-fold with 20 mM Tris-Cl, pH 7.5, 2 mM MgSO$_4$, and 0.1 mM EDTA prior to assay. Protein concentrations were estimated using an $E_{280}$ = 7.55 for the native enzyme (12) or a Coomassie blue assay (13) for the modified proteins with native enzyme as a standard.

Unless stated otherwise, ultraviolet photolysis was performed at room temperature in a magnetically stirred quartz cuvette positioned 4.5 cm from a Mineralight UVSL 25 (254 nm) ultraviolet lamp. The sample contained 0.7 mg/ml (5 μM) of enzyme, 20 mM Tris-Cl, pH 7.5, 20 mM KCl, 0.05 mM EDTA, 50 μM 2-azido-[8-3H]AMP (1666 cpm/nmol), and 140 μM Fru-1,6-P$_2$. The Fru-1,6-P$_2$ was the last addition before the photolysis. The covalent incorporation of 2-azido-

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§ Recipient of United States Public Health Service Postdoctoral Fellowship AM 06043.

$^1$The abbreviations used are: 8-N$_2$AMP, 8-azidoadenosine 5'-monophosphate; Fru-1,6-P$_2$, d-fructose 1,6-bisphosphate; 2-azido-AMP and 2-N$_2$AMP, 2-azidoadenosine 5'-monophosphate; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid); SDS, sodium dodecyl sulfate; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; NMP, nucleoside 5'-monophosphate.
0.1 mM EDTA, 0.5 mM dithiothreitol) and passage through an Affigel G-50 fine column (1.2 man GF/C glass fiber discs (14). Fully modified enzyme was isolated after a 2-min photolysis by immediate gel filtration on a Sephadex HC10/0.1 mM EDTA, pH 7.5.

The binding of [U-14C]fructose 1,6-bisphosphate and [3-3H]AMP (Amersham Corp.) to the native and fully modified enzymes were performed using the method of Hummel and Dreyer (16). The effect of IMP on [14C]fructose 1,6-bisphosphate binding to native fructose 1,6-bisphosphatase was measured at room temperature by the continuous flow dialysis method (17, 18) utilizing a 1-cm diameter chamber with a 0.16-ml lower chamber volume. Fractions (1.2 ml) were collected at a flow rate of 14 ml/h, and 1-ml aliquots were counted in 10 ml of Aquasol (New England Nuclear).

2-Azido-[8-3H]adenosine was synthesized via the method of Schaeffer and Thomas (19) using 0.25 mCi of 2-chloro-[8-3H]adenosine (Moravek Biochemicals) and 50 mg of 2-chloroadenosine (Sigma) as the starting materials. The nucleoside was phosphorylated to the 5'-monophosphate with phosphoryl chloride in trimethylphosphate (4), evaporated to dryness several times from water and methanol to remove excess volatile salts and 3H2O. The product migrated as a single spot (Rf 0.64) on thin layer cellulose plates using an isobutanol/ammonium hydroxide/acetonitrile (85:15, v/v) solvent system. The analog concentration was determined spectrophotometrically using an extinction coefficient of 15,500 M⁻¹cm⁻¹ in methanol at 271 nm. Except where indicated, the analog was added as a methanolic solution immediately before analysis and the azidotetrazole isomer ratio was four to one.

The 3-N3AMP was synthesized as described previously (21). High-performance liquid chromatography on a RP-8 (Hewlett-Packard) reverse phase column with a 0.05 M ammonium phosphate, pH 6.5/acetoni­trile (85:15, v/v) solvent was used to remove residual traces of AMP. Similar analysis indicated that the 2-azido-AMP contained no detectable adenine nucleotides.

RESULTS

2-Azido-AMP Is an Allosteric Inhibitor of Fructose 1,6-Bisphosphatase—The analog 2-azido-AMP was found to be a reversible allosteric inhibitor of fructose 1,6-bisphosphatase in the absence of ultraviolet light (Fig. 1). The enzyme's apparent affinity for the analog (K, 4.9 μM) was higher than for AMP (K, 13.7 μM) and the Hill coefficient (Fig. 1, inset) was increased (nH = 2.7 and 2.0, respectively). Since the K, for 2-azido-AMP was two orders of magnitude lower than the K, reported for 8-N3AMP (0.48 mM) with the same enzyme (4), the tight binding of 2-azido-AMP adds support to NMR data (3) which indicate that the nucleotide is bound at the allosteric site in an anti conformation. In contrast to the results obtained by others (4), highly purified 8-N3AMP inhibited the enzyme activity only 9% at a concentration of 1.2 mM. A slight (∼3%) contamination with AMP, a synthetic precursor of 8-N3AMP, may account for the allosteric inhibition of several fructose 1,6-bisphosphatases by the 8-substituted derivative (4).

2-Azido-AMP Specifically Modifies the Allosteric Site—Exposure of the 2-azido-AMP-enzyme complex to actinic light (254 nm) resulted in the covalent incorporation of the analog into the protein and the concomitant inactivation of enzyme activity (Fig. 2). The time courses for the photoincorporation and the irreversible loss of activity were found to be identical (Fig. 2A) and to parallel closely the photolytic degradation of the analog in solution. No incorporation of the label or irreversible inactivation of the enzyme was observed in the absence of the UV light. An analysis of the stoichiometry of the process (Fig. 2B) indicated that the enzyme was fully inhibited upon covalent incorporation of 4.4 mol of analog/mol of enzyme (1.1 mol of analog/subunit). Prior denaturation of the enzyme with sodium dodecyl sulfate resulted in the subsequent photoincorporation of ∼0.4 mol of analog/mol of enzyme. Therefore, approximately 10% of the incorporated radioactivity was due to nonspecific labeling while the remainder resulted in the inactivation of the enzyme with a stoichiometry equal to the subunit concentration.

The specificity of the photoaffinity labeling was examined further by photolyzing in the presence of other ligands (Table 1). The presence of AMP in the photolysis reaction mixture decreased the irreversible incorporation of the analog and protected the enzyme from inactivation. In contrast, the presence of IMP or adenosine, which are not allosteric inhibitors of the enzyme (2), had no effect on either process. It is important to note that all photolysis experiments were performed in the presence of saturating concentrations (0.14 mM) of the substrate which was found to protect the enzyme from UV-induced damage that occurred in the absence of 2-azido-AMP (results not shown). The presence of Fru-1,6-P2 had no effect on the analog photoincorporation or the resultant enzyme inactivation which suggested that modification occurred at the allosteric and not the catalytic sites. The 2-azido-AMP, therefore, fulfills the criteria for a site-directed photoaffinity probe (22) and specifically modifies the allosteric sites of the fructose 1,6-bisphosphatase with concurrent inactivation of the enzyme.

Direct binding studies were performed to compare the abilities of the native and modified enzymes to bind Fru-1,6-P2 and AMP (Fig. 3). Since a mixed enzyme population might yield ambiguous results, fructose 1,6-bisphosphatase fully modified with 2-azido-AMP was isolated by exploiting the
appropriate, optimal concentrations (as determined in the DTNB titrations, see below) of unlabeled Fru-1,6-P₂ or AMP were added to the media to decrease the nonspecific binding of [³H]AMP and [¹⁴C]Fru-1,6-P₂, respectively. Saturating concentrations of AMP or Fru-1,6-P₂ mutually increase the affinity of the pig kidney enzyme for each other (24). Additionally, as indicated by the linearity of the binding plots (24), the binding of AMP or Fru-1,6-P₂ is not cooperative even in the presence of the other ligand under the equilibrium binding conditions. The ability of the fully modified enzyme to bind Fru-1,6-P₂ clearly showed that the inactivation of the enzyme was not due to the disappearance of the substrate binding site.

The irreversible inactivation of the fructose 1,6-bisphosphatase also was not due to the dissociation of the tetrameric enzyme. Gel filtration chromatography on Sephadex G-100 indicated that the native enzyme and enzyme modified with 2-azido-AMP eluted at the same position with no evidence of dimers or monomers (results not shown). While the mechanisms of allosteric inhibition of the fructose 1,6-bisphosphatase remain obscure, these results suggest that the inactivation of the enzyme upon modification of its allosteric sites with 2-azido-AMP may be due to conformational changes similar to those induced by AMP binding.

**Titration of Thiols with DTNB As a Conformational Probe**

Titrations of various fructose 1,6-bisphosphatases with DTNB have shown that there is one highly reactive cysteine residue per subunit (25–28) which is not required for catalytic activity (25, 26). Since its rate of modification varies in the presence of specific ligands (26–28), the reactivity of this cysteine was utilized as an indicator of the enzyme's conformational changes. The behavior of the highly reactive sulfhydryl was quantified by determining the apparent second order rate constants for reaction of DTNB with the enzyme in a 4:1 ratio (DTNB equimolar to the subunit concentration).

AMP has been observed to increase the enzyme's sulfhydryl reactivity (26, 27). As shown in Fig. 4, the effect of AMP was found to be biphase; the cysteine reactivity was markedly stimulated at concentrations up to 50–100 μM AMP but declined at higher concentrations. The initial stimulatory phase was specific for AMP (Kₐ 10–20 μM); neither IMP (50 or 500 μM) nor 8-N-AMP (500 μM) were found to enhance

## Table I

**Specificity of incorporation of 2-azido-AMP**

<table>
<thead>
<tr>
<th>Additions</th>
<th>UV</th>
<th>Activity</th>
<th>Incorporation</th>
</tr>
</thead>
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<tr>
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<td>86.2</td>
<td>0.89</td>
</tr>
<tr>
<td>0.10 mM AMP</td>
<td>+</td>
<td>95.8</td>
<td>0.68</td>
</tr>
<tr>
<td>0.15 mM AMP</td>
<td>+</td>
<td>96.3</td>
<td>0.54</td>
</tr>
<tr>
<td>0.05 mM Ado</td>
<td>+</td>
<td>61.2</td>
<td>1.94</td>
</tr>
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<td>65.2</td>
<td>1.95</td>
</tr>
<tr>
<td>0.15 mM Ado</td>
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</tr>
<tr>
<td>0.05 mM IMP</td>
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<td>0.15 mM IMP</td>
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<td>64.3</td>
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<td>0.5% SDS</td>
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<td>63.4</td>
<td>0.22</td>
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</table>

## Fig. 2

Correlation between fructose 1,6-bisphosphatase inactivation and photoincorporation of 2-azido-AMP. A. Time course. O—O, per cent activity remaining; —, mol of analog incorporated per mol of enzyme. Photolysis was performed as described under "Materials and Methods" utilizing 50 μM 2-azido-[³H]AMP (1660 cpm/nmol) and 0.7 mg/ml (5 PM) of enzyme. At various times the ultraviolet light path was interrupted by interposing an opaque filter and aliquots were either acid precipitated for the measurement of incorporation or diluted 10-fold with cold Buffer A containing 0.1 mg/ml of bovine serum albumin. The samples were further diluted in 20 mM Tris-Cl, 2 mM MgSO₄, 0.1 mM EDTA, pH 7.5, and the enzyme activity was determined using an inorganic phosphate release assay. The total enzyme dilution in the assay was 5000-fold. B. Stoichiometry. The data in A were replotted and extrapolated to total inactivation of the enzyme.

### Notes

* Photoaffinity Labeling of Fructose 1,6-Bisphosphatase
* Specificity of incorporation of 2-azido-AMP
* Titration of Thiols with DTNB As a Conformational Probe
* Inability of the occupied nucleotide sites to bind to a Cibacron blue F₃GA affinity column (23). This fully modified enzyme had a specific activity 1–3% of the native form and contained 3.8–4.3 mol of analog/mol of enzyme.

The binding of AMP to the native and fully modified enzymes was markedly different (Fig. 3A). Under conditions where the native enzyme bound 3.36 mol of AMP, the fully modified enzyme bound only 0.14 mol of AMP per mol of enzyme demonstrating further that the photoaffinity analog modified the allosteric sites. In contrast, the fully modified enzyme was capable of binding the substrate fructose 1,6-bisphosphate (Fig. 3B). Under conditions where the native enzyme bound 2.40 mol of substrate per mol (60% saturation), the fully modified enzyme was capable of binding 1.96 mol of Fru-1,6-P₂ per mol (49% saturation). Note that, where appropriate, optimal concentrations (as determined in the DTNB titrations, see below) of unlabeled Fru-1,6-P₂ or AMP were added to the media to decrease the nonspecific binding of [³H]AMP and [¹⁴C]Fru-1,6-P₂, respectively. Saturating concentrations of AMP or Fru-1,6-P₂ mutually increase the affinity of the pig kidney enzyme for each other (24). Additionally, as indicated by the linearity of the binding plots (24), the binding of AMP or Fru-1,6-P₂ is not cooperative even in the presence of the other ligand under the equilibrium binding conditions. The ability of the fully modified enzyme to bind Fru-1,6-P₂ clearly showed that the inactivation of the enzyme was not due to the disappearance of the substrate binding site.

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<td>0.5% SDS</td>
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<td>63.4</td>
<td>0.22</td>
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</table>
FIG. 3. Comparison of binding properties of native and fully modified fructose 1,6-bisphosphatases. Binding was performed at room temperature using Sephadex G50-fine columns (1 × 25 cm) and 0.7 mg of native (O—O) or fully modified enzyme (■—■) that was prepared as described under “Materials and Methods” using unlabeled 2-azido-AMP. A, [3H]AMP binding. The columns and samples (1 ml) were equilibrated in Buffer A containing 8 μM [3H]AMP (771 cpm/nmol) and 140 μM Fru-1,6-P₂. B, [14C]fructose 1,6-bisphosphate binding. The columns and samples (1 ml) were equilibrated in Buffer A containing 10.9 μM [14C]fructose 1,6-bisphosphate (780 cpm/nmol), and in the case of the native enzyme, 50 μM AMP. Vₑ = volume of eluate, Vᵣ = volume of column.

the rate of the DTNB reaction (results not shown). The decline in cysteine reactivity at high AMP concentrations was found to be nonspecific relative to the nucleotide. In the presence of an optimal concentration of AMP (50 μM), IMP caused a decrease in the second order rate constant comparable to the decline observed at high AMP concentrations. A similar decrease was found to occur upon the addition of high concentrations of 8-N₃AMP in the presence of 50 μM AMP (results not shown). As the initial stimulation in cysteine reactivity demonstrated a concentration dependence and specificity comparable to the AMP-induced inhibition of the enzyme, the increase in the second order rate constant apparently resulted from conformational changes induced upon binding of AMP to the allosteric site.

Due to its low specificity and affinity, the decrease in sulfhydryl reactivity at high AMP concentrations may have been the result of conformational changes induced by nonspecific binding of the nucleotide at the fructose 1,6-bisphosphatase binding site. In the presence of 50 μM AMP, IMP (Fig. 5) was found to be a competitive inhibitor (Kᵢ = 1.26 mM) of \([14C]\text{Fru-1,6-P₂}\) binding (Kᵢ = 10.5 μM). Since 8-N₃AMP mimicked only IMP in the alteration of sulfhydryl reactivity, we suggest that studies on fructose 1,6-bisphosphatases using high nucleotide concentrations (>10 × Kᵢ) be reinterpreted in consideration of nonspecific interactions at the active site.

Fully Modified Enzyme Mimics the Enzyme-AMP Complex—The apparent response of the sulfhydryl reaction rates to both specific and nonspecific nucleotide interactions enabled a comparison between the native enzyme and enzyme fully modified at its allosteric sites with 2-azido-AMP. Preliminary experiments indicated that modification of the fructose 1,6-bisphosphatase with the analog affected neither the total number nor the number of highly reactive cysteines (four).

As shown in Table II, the second order rate constant for the reaction of DTNB with the fully modified enzyme was 6-fold higher than that observed for the native enzyme in the
absence of added ligands. A similar (4-fold) increase in thiol reactivity was observed for the native enzyme in the presence of optimal concentrations of AMP. Additionally, the sulphydryl reactivity of the fully modified enzyme responded to nucleotides in a manner consistent with the nonspecific binding observed in the native enzyme (see above). The addition of either AMP or IMP to the fully modified enzyme resulted in decreases in the DTNB reaction rate comparable to those described above for the native enzyme (Table II). Addition of lower concentrations of AMP or IMP resulted in proportionately lower inhibitions of the sulphydryl reactions; no increase in sulphydryl reactivity was observed upon the addition of nucleotides to the fully modified enzyme. The magnitudes and specificities of the changes in thiol reactivity suggest that the fully modified enzyme has a similar conformation to the native enzyme saturated at its allosteric sites with AMP.

The addition of substrate has been observed to inhibit the reaction of DTNB with the highly reactive cysteine residues of the native and fully modified enzymes. The conditions were identical with Fig. 4.

As shown in Table III, a 1.5-fold enhancement in thiol reactivity was observed when Mg\(^{2+}\) was added to the fully modified enzyme in the absence of added nucleotides. The addition of Mg\(^{2+}\) to the native enzyme resulted in a similar enhancement (2.5-fold) in the presence of 50 \(\mu\)M AMP but had no effect on the DTNB reaction in the absence of the nucleotide. The possibility that increased rates of reactivity resulted from the release of the second mol of the 5-thio-2-nitrobenzoic acid via disulfide formation was examined. In each case the expected total absorbance at 412 nm was observed after the stoichiometric titration with DTNB and the subsequent addition of dithiothreitol doubled the absorbance as expected. Identical results were obtained in a few cases where substoichiometric ratios (0.5:1) of DTNB to enzyme subunit were used. Additionally, the presence of ligands had no effect on the number of fast reacting cysteines (four) (measured in the presence of excess DTNB) in both the native and fully modified enzymes except in the case of Fru-1,6-P2, where the rates of DTNB reaction were too slow to distinguish the number of reacting sulphydryls.
Photoaffinity Labeling of Fructose 1,6-Bisphosphatase

TABLE III
Changes in the allosteric constants for AMP according to the extent of modification

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<thead>
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<th>(n_H)</th>
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<td>2.9</td>
<td>27.5</td>
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</table>

* Incorporation: mol of analog covalently bound per mol of enzyme.

\(K_h\) is the AMP concentration that gives 50% of the activity of the enzyme in the absence of the inhibitor.

\(n_H\) (Hill coefficient) is the slope of the line in the Hill plot.

The kinetic behavior of the half-modified enzyme suggests that cooperative effects in the pig kidney fructose 1,6-bisphosphatase may occur during the binding of only the first one or two molecules of AMP. Since the extents of modification represented an average over the entire enzyme population and low levels of modification yielded intermediate results (Fig. 6, Table III), it was not possible to determine unequivocally whether the loss of allosteric behavior occurred after the occupancy of the first or second site. Note that the changes in the Hill coefficient upon partial modification also indicated that the photoincorporation of 2-azido-AMP into an enzyme subunit did not inactivate the entire tetramer. Since the loss of enzyme activity was proportional to the extent of analog incorporation (Figs. 2B and 6), the modification of an allosteric site inactivated the associated catalytic site without an effect on the maximum velocity of the adjacent subunits.

CONCLUSIONS

The photoaffinity analog 2-azido-AMP closely mimicked the allosteric interactions of AMP with the pig kidney fructose 1,6-bisphosphatase. Photoaffinity labeling studies indicated that the analog specifically incorporated into the enzyme's allosteric sites with high efficiency and appropriate stoichiometry. The resulting modified enzyme was found to be irreversibly inactivated, but not via subunit dissociation or loss of the fructose 1,6-bisphosphate binding site.

Titrations of the native enzyme's fast reacting cysteine residues suggested that four enzyme conformations could be distinguished by their relative rates of reaction: (I) \(E(M^2)\), \(k = \frac{1}{2}\); (II) \(E(AMP)\), \(k = 5\); (III) \(E(AMP)M^2\), \(k = 10\); and (IV) \(E(Fru-1,6-P)\), \(k = 0.4\). Additional studies suggested that AMP also may interact at the fructose 1,6-bisphosphate binding site. Since IMP and 8-N3AMP appeared to mimic only the nonspecific effects of AMP, the site of modification in previous studies (4) remains in doubt.

Enzyme fully modified with 2-azido-AMP demonstrated sulfhydryl reaction rates consistent with conformations attained by the native enzyme in the presence of AMP (II-IV). The results suggest that the covalently incorporated analog behaved like nondissociable AMP and inactivated the enzyme via conformational changes similar to those induced by AMP.

Fructose 1,6-bisphosphatase partially modified with 2-azido-AMP showed a dramatic loss of enzyme cooperativity toward inhibition by AMP (50% modified, \(n_H = 1\)) without affecting the apparent inhibition constant for AMP at the unmodified subunits. While the modified enzyme consisted of a mixed population, changes in the Hill coefficient indicated that catalytically active partially modified enzyme species were present. Since the photoaffinity labeling was performed under noncooperative binding conditions, it is unlikely that discrimination against particular enzyme forms would occur at the time of modification. If the partially modified enzyme mimics the native enzyme, the allosteric behavior of fructose 1,6-bisphosphatase toward AMP probably is restricted to the occupancy of at most the first two sites without alterations in the affinity of the enzyme for AMP. Further studies will be required to more fully characterize the kinetic and allosteric behavior of the partially modified enzyme and to compare these with fructose 1,6-bisphosphatases from other sources.

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