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Covalent Modification of the Inhibitor Binding Site(s) of *Escherichia coli* ADP-Glucose Synthetase: Specific Incorporation of the Photoaffinity Analogue 8-Azidoadenosine 5′-Monophosphate†

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ABSTRACT: The photoaffinity agent 8-azidoadenosine 5′-monophosphate (8-N_3AMP) is an inhibitor site specific probe of the *Escherichia coli* ADP–glucose synthetase (ADPG synthetase). In the absence of light, 8-N_3AMP exhibits the typical reversible allosteric kinetics of the physiological inhibitor AMP. In the presence of light (254 nm), the analogue specifically and covalently modifies the enzyme, and photoincorporation is linearly related to loss of catalytic activity up to at least 65% inactivation. The substrate ADPG provides nearly 100% protection from 8-N_3AMP photoinactivation, while the substrate ATP provides approximately 50% protection and the inhibitor AMP, approximately 30% protection. These three adenylate allosteric effectors of *E. coli* ADPG synthetase also protect it from photoincorporation of 8-N_3AMP. A structural overlap of the inhibitor and substrate binding sites is proposed which explains the protection data in light of the known binding and kinetic properties of this tetrameric enzyme.

Adenosine diphosphate glucose (ADPG)[1] synthetase (EC 2.7.7.27) catalyzes the first committed reaction in the metabolic synthesis of glycogen in *Escherichia coli*, ATP + α-glucose-1-P $\rightleftharpoons$ ADPG + PP_i (Preiss, 1973, 1978). An allosterically regulated enzyme, its physiological activator is Fru-P_2, and it is inhibited by AMP, ADP, and P_i (Gentner et al., 1969; Govons et al., 1973; Haugen et al., 1974; Preiss et al., 1966). The complete amino acid sequence of this enzyme was deduced by sequencing of various CNBr peptides and complete nucleotide sequencing of the structural *glg* C (Baecker et al., 1983). The enzyme consists of four identical subunits with a molecular weight each of 48 762 (Baecker et al., 1983; Haugen et al., 1976).

In previous chemical modification studies, the allosteric activator pyridoxal phosphate was used as an affinity label to

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[1] Abbreviations: 8-N_3AMP, 8-azidoadenosine 5′-monophosphate; 8-N_3ATP, 8-azido-ATP; 8-N_3ADPG, 8-azido-ADP-glucose; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Fru-P_2, fructose 1,6-bisphosphate; BPA, bovine plasma albumin; EDTA, ethylenediaminetetraacetic acid; ADPG, adenosine diphosphate glucose; DTE, dithioerythritol; HPLC, high-performance liquid chromatography; MSAP, mouse submaxillary arginyl protease; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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identify the activator binding site near the N-terminus, Lys-38 (Parsons & Preiss, 1978a,b). Also identified was a pyridoxal phosphate modified peptide representing a second site, a putative substrate binding site at Lys-194, that was protected from modification by Mg^{2+} and ADPG.

A previous binding study of the substrates and effectors of *E. coli* ADPG synthetase showed that ATP binds to two sites per tetramer in the absence of glucose-1-P (Haugen & Preiss, 1979). CrATP, a nonreactive analogue of ATP, also binds at two sites in the absence of glucose-1-P, but to four sites in its presence. AMP and ADPG bind to four sites per tetramer. The study proposed an enzyme structure containing three separate functional binding sites per subunit, one each for substrates, activator, and inhibitor.

AMP, the allosteric inhibitor, affects the ATP saturation curve by increasing the $S_{0.5}$ of ATP and the Hill slope value, n, as well as by decreasing the V_{max} of *E. coli* ADPG synthetase (Gentner & Preiss, 1968; Preiss, 1978). These multiple kinetic effects suggest only partially competitive inhibition. To identify the inhibitor binding site, and to understand better the allosteric phenomena and the complicated inhibitor kinetic properties, the photoaffinity labeling analogue 8-N_3AMP was used as a site-specific probe of the *E. coli* ADPG synthetase inhibitor binding site(s).

Experimental Procedures

Materials

Chemicals. [2-^{3}H]-8-N_3AMP (16.0 Ci/mmol) was obtained from New England Nuclear. [^{14}C]-8-N_3AMP (34.0 mCi/mmol) was the generous gift of B. E. Haley, The University of Wyoming. [U-^{14}C]Glucose-1-P (294 mCi/mmol) was from Amersham/Searle. Adenine, adenosine, 5′-AMP, 8-N_3AMP, ATP, ADPG, and guanidine hydrochloride were all purchased from Sigma. All other chemicals were of the highest possible commercial grade. Thin-layer cellulose chromatograms were obtained from Kodak (13255–w/o fluorescent indicator).

Photoaffinity Analogue Purity. 8-N_3AMP routinely migrated as a single spot (R_F 0.45) on thin-layer cellulose plates in a 1-butanol/acetic acid/water (5:2:3 v/v) solvent system. 8-N_3AMP in water at neutral pH gave the characteristic UV spectra (Haley, 1977), with a λ_{max} of 280–281 nm. The λ_{max} was completely abolished by preirradiation with 254-nm light. The concentration of 8-N_3AMP was determined spectrophotometrically by using an extinction coefficient of 13 300 M^{-1} cm^{-1} in water at 281 nm (Muneyama et al., 1971).

Purification of ADPG Synthetase. The enzyme was purified from *E. coli* B, strain AC7OR1/pOP12 (Okita et al., 1981) cells, as previously described (Haugen et al., 1974; Okita et al., 1981). Protein concentrations were measured by the absorbance at 280 nm, using an extinction coefficient of 1.0 per 1.0 mg of protein mL^{-1} cm^{-1} (Haugen et al., 1976). All ADPG synthetase concentrations refer to concentrations of subunit (M_r 48 762).

Methods

ADPG Synthetase Assay (Synthesis Direction): Activated Conditions. Synthesis of ADP-[^{14}C]glucose from [U-^{14}C]-glucose-1-P and ATP was routinely measured as previously described (Ghosh & Preiss, 1966). The reaction mixture contained 20 μmol of HEPES (pH 7.0), 100 μg of BPA, 0.1 μmol of [U-^{14}C]glucose-1-P (1000 cpm/nmol), 0.3 μmol of ATP, 1.0 μmol of $MgCl_2$, 0.2 μg of yeast inorganic pyrophosphatase, 0.3 μmol of Fru-P_2, and enzyme in a total volume of 0.2 mL. The reaction was initiated by addition of enzyme and assayed at 37 °C for 10 min. One unit of enzyme activity is that amount which will catalyze the production of 1 μmol of ADP-[^{14}C]glucose/min at 37 °C.

Inhibition Kinetic Studies. Initial velocity kinetic experiments using 5′-AMP or 8-N_3AMP as inhibitors of ADPG synthetase were performed in the dark in the synthesis direction as described previously (at two different Fru-P_2 concentrations) with varying concentrations of inhibitor added to the reaction mixture. Ten microliters of enzyme solution (10 μg/mL) was utilized for the assay.

Treatment of Data To Obtain Kinetic Constants. Kinetic data are plotted as velocity vs. inhibitor concentration and are replotted as a Hill plot (Changeux, 1963; Hill, 1910). V_{max} is the velocity under saturating activated conditions in the absence of inhibitor. The $I_{0.5}$ determined from the Hill plots corresponds to the concentration of inhibitor required for half-maximal inhibition (Koshland et al., 1966); n, the Hill constant or interaction coefficient, is a measure of the degree of sigmoidicity of the original saturation curve (Hill, 1910; Levitzki & Koshland, 1969; Taketa & Pogell, 1965).

Photoaffinity Modification. (A) Photoinactivation with Unlabeled 8-N_3AMP. Enzyme (0.1–6 nmol of subunit) was incubated in the dark at room temperature for 10 min with 0.5 mM 8-N_3AMP (except for 8-N_3AMP saturation experiments, in which a range of concentrations was used) in 50 mM Tris-HCl (pH 7.2) containing 50 μM Fru-P_2 unless specified otherwise. The reaction mixture, a total volume of 0.2 mL, was placed in a Coors spot plate well and irradiated at room temperature with a Mineral-light Model UVS-54 (254 nm) lamp at a distance of 30 cm. Aliquots of the reaction mixture were removed prior to irradiation and, at various times during irradiation, diluted at least 50-fold to displace any reversibly bound inhibitor in 50 mM HEPES (pH 7.0) at 0.1 mg/mL BPA and 2.5 mM DTE and assayed for enzyme activity in the synthesis direction. In protection experiments, an enzyme effector or other ligand was added to the reaction mixture to give the indicated final concentration.

(B) Photoincorporation of Labeled 8-N_3AMP. The reaction conditions were essentially the same as described previously for photoinactivation except that up to 100 nmol of enzyme subunit was used in a total volume of 0.25–1.5 mL.

(C) Isolation of Enzyme after 8-N_3AMP Photoincorporation. To remove noncovalently bound label, radioactive samples were made 6 M in guanidine hydrochloride and either (a) dialyzed twice (6–8 h each time) against 100 volumes of 50 mM Tris-HCl (pH 7.2) buffer containing 1 mM EDTA and 50 mM KCl and then twice again against 100 volumes of the same buffer containing 6 M guanidine hydrochloride, (b) dialyzed 4 times against 100 volumes of 50 mM Tris-HCl (pH 7.2) buffer containing 1 mM EDTA and 0.5 mM DTE, or (c) applied to a Sephadex G-50 (fine) column equilibrated with 50 mM Tris-HCl (pH 7.2) buffer containing 1 mM EDTA, 0.5 mM DTE, and 6 M guanidine hydrochloride. Aliquots were counted for radioactivity and assayed for protein by a modification of the Lowry (1951) method. The three methods for removing noncovalently bound label were equally effective (essentially 100%) as determined by the absence of radioactivity in treated samples which had been incubated but not photolyzed with radioactive 8-N_3AMP.

Results

Allosteric Inhibition Kinetics. Figure 1 shows the effect of two different Fru-P_2 concentrations on 5′-adenylate and 8-N_3AMP reversible allosteric inhibition of *E. coli* ADPG synthetase in the absence of ultraviolet light. 8-N_3AMP exhibits the typical sigmoidal inhibition kinetics of AMP at the two different activator concentrations. From the corresponding

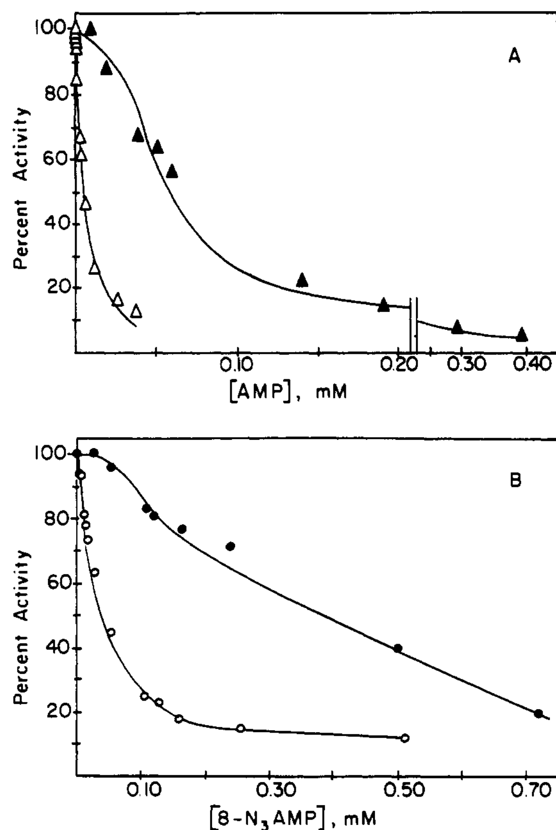

FIGURE 1: Inhibition of ADPG synthetase by 8-N_3AMP and AMP. (A) AMP inhibition in the presence of 50 μM Fru-P_2 (▲) or 1.5 mM Fru-P_2 (△). (B) 8-N_3AMP inhibition in the presence of 50 μM Fru-P_2 (●) or 1.5 mM Fru-P_2 (○). Enzyme activity was assayed as described under Experimental Procedures.

Table I: Allosteric Inhibition Kinetics of *E. coli* ADPG Synthetase[a]

inhibitor	[Fru-P_2]	$I_{0.5}$ (μM)	n
AMP	50 μM	5.9	1.3
	1.5 mM	64	1.7
8-N_3AMP	50 μM	43	1.1
	1.5 mM	307	1.7

[a] The calculated kinetic values for AMP and 8-N_3AMP inhibition were obtained from Hill plots from the data presented in Figure 1.

Hill plots (not shown), one obtains $I_{0.5}$ values for 8-N_3AMP that are somewhat higher than those for AMP, but the $I_{0.5}$'s for both shift to higher concentrations when the Fru-P_2 concentration is increased (Table I). As also shown in Table I, the strength of positive interaction among sites for 8-N_3AMP is nearly identical with the AMP site interaction at both activator concentrations. When 0.11 mM 8-N_3AMP was included in the reaction mixtures, the $I_{0.5}$ for AMP at 1.5 mM Fru-P_2 decreased from 64 to 54 μM and the Hill coefficient decreased from 1.7 to 1.3 (data not shown). These effects are expected for two inhibitors binding at the same site(s) in an allosteric system exhibiting positive cooperativity.

Photoinactivation of ADPG Synthetase by 8-N_3AMP. Photolysis of 8-N_3AMP in the presence of *E. coli* ADPG synthetase resulted in the rapid and irreversible loss of enzyme activity. About 70% of the activity was lost in 5 min under the conditions described under Experimental Procedures. About 90% activity was lost in 15 min. Essentially no loss of enzyme activity occurred in the absence of 8-N_3AMP, and in a control experiment, there was no irreversible inactivation of enzyme in the absence of ultraviolet light. The same rate of photoinactivation was observed for all enzyme concentrations used (0.5–30 μM subunit). As shown in Figure 2, concentrations of 8-N_3AMP in the range of its reversible $I_{0.5}$ values were effective for photoinactivation at two different activator concentrations.

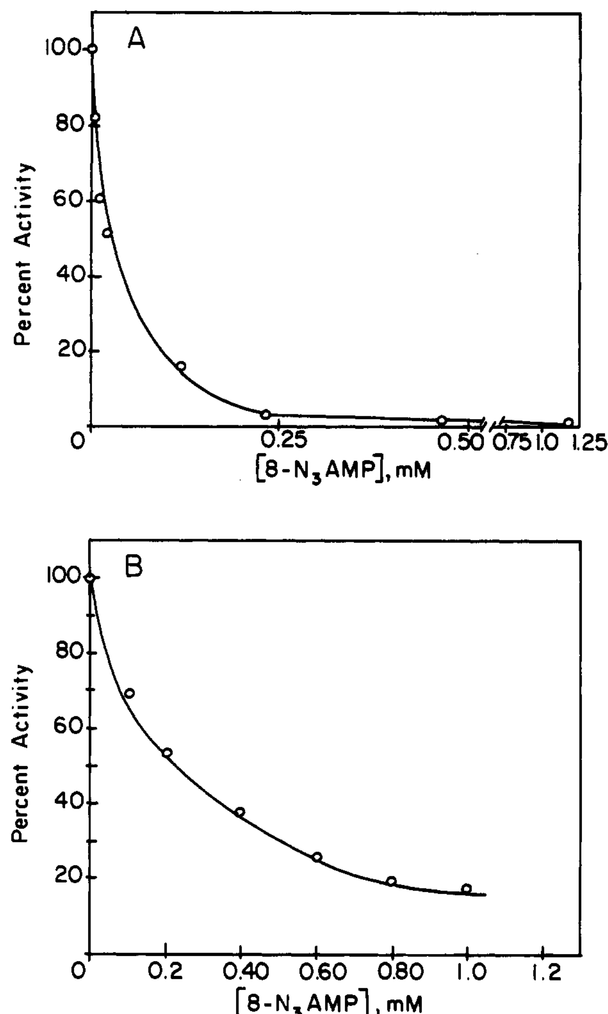

FIGURE 2: Photoinactivation of ADPG synthetase as a function of initial 8-N_3AMP concentration at two different activator concentrations. (A) 25 μg/mL (0.5 μM) enzyme was photoinactivated in the presence of 50 μM Fru-P_2 for 15 min. (B) 0.5 mg/mL (10 μM) enzyme was photoinactivated in the presence of 1.0 mM Fru-P_2 for 3 min.

Table II: Effector Protection of ADPG Synthetase Photoinactivation by 8-N_3AMP[a]

compound	concn (mM)	% residual act.
none		0
adenine	2.0	20
5'-AMP	1.9	33
ATP	2.1	50
P_i	10	0
5'-AMP + P_i	1.9 + 10	57
ADPG	2.0	92

[a] 25 μg/mL (0.5 μM) enzyme was photoinactivated for 15 min as described under Experimental Procedures.

ADPG synthetase was photoinactivated with 8-N_3AMP in the presence of various enzyme substrates and inhibitors to demonstrate further the photoaffinity specificity. Table II shows that ATP and ADPG specifically protect the enzyme from photoinactivation over the nonspecific ultraviolet shielding provided by adenine. AMP protects only slightly over the nonspecific protection. The presence of P_i, however, enhances the protection by AMP, possibly filling the larger substrate site, which suggests that the substrate binding site is also involved in 8-N_3AMP binding. Figure 3 shows the dependence

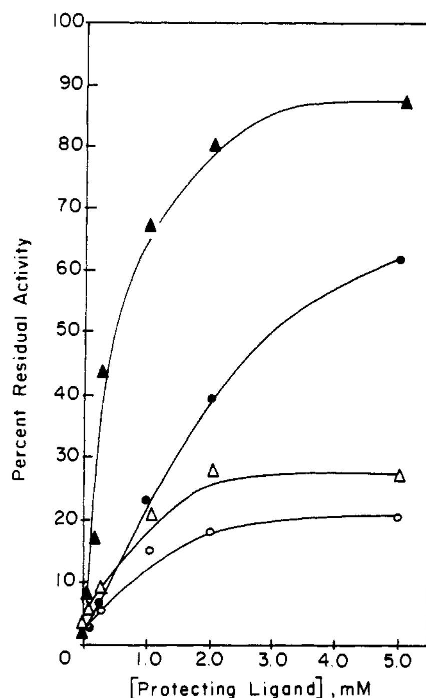

FIGURE 3: Adenylate ligand protection of 8-N_3AMP photoinactivation of ADPG synthetase. 0.5 mg/mL (10 μM) enzyme was photolyzed for 20 min. ADPG (▲), ATP (●), AMP (△), and adenosine (○) were used as described under Experimental Procedures to protect against photoinactivation. $MgCl_2$, 5 mM, was included in all reaction mixtures.

of the protection on the nucleotide ligand concentration. The hyperbolic shape of the curves suggests that the nucleotides protect by binding to the enzyme and not by screening the photolytic reactions from ultraviolet light. ADPG is by far the best protector, followed by ATP and AMP. The fact that protection is enhanced with a larger molecular size of protector suggests that there may be some site overlap between the inhibitor and substrate binding sites or that 8-N_3AMP is actually binding at the substrate site.

Covalent Incorporation of 8-N_3AMP into ADPG Synthetase. Photoinactivation of ADPG synthetase with [^{14}C]-8-N_3AMP followed by gel filtration on Sephadex G-50 in the presence of guanidine hydrochloride showed that the reagent covalently binds to the enzyme upon photoinactivation (Figure 4). The peak of protein activity in Figure 4A coincides with a peak of radioactivity that is resolved from the unbound radioactive reagent. A dark control experiment (Figure 4B) showed that there is no incorporation of label in the absence of ultraviolet light. The time courses for photoincorporation and photoinactivation are identical (not shown). Photolytic incorporation of [2-^{3}H]-8-N_3AMP is linearly related to loss of enzyme catalytic activity up to at least 65% inactivation (10-min photolysis) and extrapolates to 0.4 mol of 8-N_3AMP covalently bound per mole of enzyme subunit (Figure 5). Complete photoincorporation, however, results in 0.8–1.0 mol of 8-N_3AMP bound per mole of subunit.

There are at least two likely explanations for this biphasic stoichiometry of incorporation. Incorporation may occur initially at a specific site per dimer, causing a conformational change that allows photoincorporation at the specific site on each of the two remaining active subunits. Alternatively, nonspecific labeling may occur in addition to the specific site per dimer. This latter explanation is unlikely, however, because comparison of reverse-phase HPLC tryptic peptide maps shows that [2-^{3}H]-8-N_3AMP binds at or near the same site(s) no matter what the level of photoinactivation (C. E. Larsen et al., unpublished results). Another indication that little, if any,

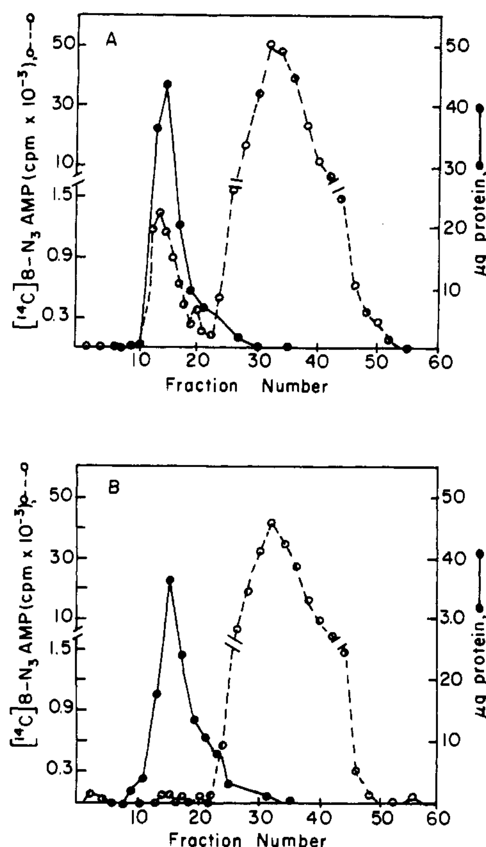

FIGURE 4: Photolytic incorporation of [^{14}C]-8-N_3AMP into ADPG synthetase. (A) 7.44 mg/mL (153 μM) enzyme and 0.5 mM [^{14}C]-8-N_3AMP (2.75 mCi/mmol) were photolyzed for 20 min in a reaction mixture of 0.625 mL. An aliquot was eluted on a Sephadex G-50 (fine) column (2 mL; 0.75 × 4.5 cm). 75-μL samples were collected at a flow rate of 15 mL/h. For additional details, see Experimental Procedures. (B) Control without photolysis but with incubation of the enzyme with [^{14}C]-8-N_3AMP in the dark for 20 min.

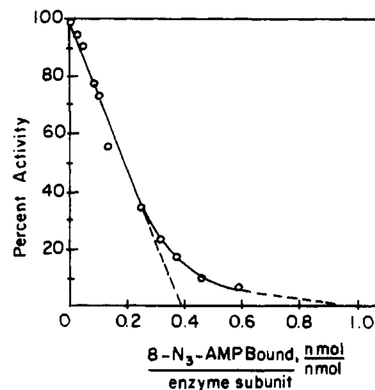

FIGURE 5: Correlation of photoinactivation with photoincorporation of [2-^{3}H]-8-N_3AMP for ADPG synthetase. Stoichiometry of photoincorporation. 2.5 mg/mL (50 μM) enzyme was photoinactivated with 0.5 mM [2-^{3}H]-8-N_3AMP (1.5 × 10^7 cpm/μmol) in 1.0-mL reaction mixtures at 1.0 mM Fru-P_2 from 0 to 45 min after preincubation. At each time point, aliquots were removed for assay of enzyme activity and for incorporation. Noncovalently bound analogue was removed from the latter by exhaustive dialysis as described under Experimental Procedures. Data were extrapolated to total enzyme inactivation.

nonspecific photoincorporation occurs is that AMP, ATP, and ADPG protect ADPG synthetase from photoincorporation of [^{3}H]-8-N_3AMP as well as from photoactivation (Table III). These results, taken together, support the specificity of covalent photoaffinity labeling of *E. coli* ADPG synthetase by 8-N_3AMP.

Table III: Adenylate Effector Protection of Photolabeling by [^{3}H]-8-N_3AMP[a]

additions	% residual act.	mol of [^{3}H]-8-N_3AMP/ mol of subunit
none	31	0.25
5 mM adenosine	68	0.17
5 mM 5′-AMP	77	0.07
5 mM ATP	100	0.04
5 mM ADPG	100	0.04

[a] 2.5 mg/mL (50 μM) enzyme was photolyzed in the presence of 0.5 mM [2-^{3}H]-8-N_3AMP (5.0×10^7 cpm/μmol) in 0.25-mL reaction mixtures with additions as indicated for 12 min. 5 mM $MgCl_2$ was included in all reaction mixtures. The solutions were dialyzed 4 times against 100 volumes of 50 mM Tris-HCl (pH 7.2) buffer containing 1 mM EDTA and 0.5 mM DTE. Aliquots were then counted for radioactivity. Complete recovery of protein was assumed.

Discussion

In the present study, 8-N_3AMP was tested as an inhibitor site directed photoaffinity labeling agent of *E. coli* ADPG synthetase. The approach was based on previously successful attempts to use 8-azido purine analogues as such agents for other nucleotide binding protens (Aiba & Krakow, 1980; Kerlavage & Taylor, 1980; Marcus & Haley, 1979; Potter & Haley, 1983; Schafer et al., 1984). The following were demonstrated in this report: (a) 8-N_3AMP exhibits the typical reversible allosteric inhibition kinetics of AMP. (b) The rate of photoinactivation follows saturation kinetics, and concentrations of 8-N_3AMP in the range of its reversible $I_{0.5}$ are effective for photoactivation. (c) The enzyme substrates ADPG and ATP specifically protect the enzyme from photoinactivation of 8-N_3AMP. The saturable nature of the concentration dependence of protection shows that the nucleotides protect by binding to the enzyme cooperatively with 8-N_3AMP. (d) 8-N_3AMP stoichiometrically labels the enzyme. Photoincorporation is linearly related to photoinactivation up to at least 65% inactivation and extrapolates to 0.4 mol of 8-N_3AMP covalently bound per mole of enzyme subunit. Complete photoincorporation, however, results in 0.8–1.0 mol of 8-N_3AMP bound per mole of subunit. (e) AMP, ATP, and ADPG protect the enzyme from photoincorporation of labeled 8-N_3AMP.

Clearly, from the results presented here, 8-N_3AMP is a specific site directed photoaffinity labeling agent for *E. coli* ADPG synthetase. However, does 8-N_3AMP bind at the inhibitor site or at the substrate site? Though the analogue mimics the inhibitor's allosteric kinetics and 8-N_3AMP concentrations in the range of its reversible $I_{0.5}$ are effective for photoinactivation, the substrates are better protectors of photoinactivation than is AMP. These somewhat contradictory results, however, along with a number of other lines of evidence, may suggest that the AMP inhibitor binding site and the ATP/ADPG substrate binding site overlap, at least to some extent.

First of all, the binding experiments mentioned previously (Haugen & Preiss, 1979) showed that ATP stimulates the binding of AMP to the *E. coli* ADPG synthetase in the absence of Fru-P_2. This was an early suggestion that there was a cooperative interaction between the inhibitor and substrate binding sites. It has also been shown that ADPG, ATP, and AMP protect *E. coli* ADPG synthetase from the substrate binding site directed analogues 8-N_3ATP and 8-N_3ADPG in the same relative order as they protect the enzyme from 8-N_3AMP (Lee et al., 1986). More directly, comparison of reverse-phase HPLC tryptic peptide maps of 8-N_3AMP, 8-N_3ATP, and 8-N_3ADPG photoinactivated enzyme shows that all three analogues bind at or near the same site(s) (C. E. Larsen et al., unpublished results). Finally, the stoichiometry of 8-N_3AMP incorporation exhibits biphasic characteristics. The early phase stoichiometry of nearly 0.5 mol of analogue bound per mole of subunit is consistent with the published binding properties between the substrate ATP and *E. coli* ADPG synthetase (Haugen & Preiss, 1979). The final stoichiometry of 0.8–1.0 mol bound/mol of subunit is consistent, however, with the binding properties between the inhibitor AMP and the enzyme.

Under this scheme, the inhibitor and substrate binding sites share a binding locus for at least part of the adenine ring. 8-N_3AMP may bind only to *one* of these adenylate effector sites on a given enzyme subunit but prefers the AMP site. This would explain the reported results. First, the allosteric kinetics are reasonable if the AMP binding site is preferred. Second, it could explain the biphasic photoincorporation stoichiometry. Complete photoincorporation results in 4 mol of 8-N_3AMP bound per mole of tetrameric enzyme, but the first 2 mol of analogue appears to bind easier and has a proportionally greater effect on enzyme inactivation. The second 2 mol of analogue has reduced binding availability because 8-N_3AMP may bind at or partially mimic binding at the ATP site, and ATP exhibits half-site binding to the enzyme (Haugen & Preiss, 1979). Only the AMP or ADPG site(s) would be fully available for the second 2 mol of analogue, and the tetramers would already be almost completely inactivated after the first 2 mol of 8-N_3AMP was bound. Third, the protection results are explainable. In the absence of protector, 8-N_3AMP binds to the AMP site primarily, but in the presence of AMP, 8-N_3AMP could still bind to a nearby substrate site and force AMP out of its inhibitor site [ATP in the presence of Fru-P_2 is known to inhibit the binding of AMP (Haugen & Preiss, 1979)]. This explains why AMP offers relatively little protection even though the ligand is inhibitor site directed. ATP, at best, only specifically protects half of the sites since it only binds to half the subunits and half of the AMP and ADPG sites are still available. ADPG gives full protection because it binds to all four subunits and cannot be forced out of its binding site by a smaller 8-N_3AMP attempting to bind at a nearby site. A simpler scheme than the one presented here, in which 8-N_3AMP binds only to the substrate binding site, fails to explain either the allosteric kinetics or the photoincorporation stoichiometry.

The most direct test of the photoaffinity analogue specificity would be structural examination of the ADPG synthetase site(s) covalently modified with labeled 8-N_3AMP. In order to locate the 8-N_3AMP binding site and to test for 8-N_3AMP specificity, we analyzed the [2-^{3}H]-8-N_3AMP labeled peptides generated by CNBr cleavage followed by MSAP digestion (Larsen et al., unpublished results). The major 8-N_3AMP binding site was identified as Tyr-113, which is located within the previously identified major 8-N_3ADPG binding region (Lee & Preiss, 1986).

Registry No. 8-N_3AMP, 60731-47-7; AMP, 61-19-8; ADPG, 2140-58-1; ATP, 56-65-5; ADPG synthetase, 9027-71-8.

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Partial Reversal of α2u Globulin Gene Expression by Thyroxine in the Liver of Diabetic Rats[†]

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ABSTRACT: Synthesis of α2u globulin and its mRNA has been used as an index to monitor the effect of thyroxine on specific gene expression in the liver of hypoinsulinemic male rats. Administration of a physiological dose of thyroxine can partially reverse (to approximately 30% of the normal control) the marked reduction (more than 90%) in the hepatic levels of α2u globulin and its mRNA during streptozotocin-induced diabetes. Estimation of newly synthesized α2u globulin RNA transcripts from the native chromatin of isolated liver nuclei by "nuclear runoff experiments" showed that thyroxine can elevate the rate of transcription of α2u globulin gene in the diabetic rat. Hypoinsulinemic diabetes is also found to be associated with an approximately 35% reduction in the thyroid hormone receptor level as compared to the normal control. The stimulatory effect of thyroxine on the synthesis of α2u globulin and its mRNA was also evident in spontaneous diabetic Wistar "BB" rats. From these studies it can be concluded that severe hypoinsulinemia can cause a decrease in thyroid hormone action at the level of specific gene expression.

Liver is an important target organ for both insulin and thyroxine. An optimum hepatic synthesis of the major urinary protein α2u globulin in the rat is known to require both of these hormones (Roy et al., 1976, 1980; Kurtz et al., 1976). In addition, androgens, glucocorticoids, and growth hormone are also involved in the regulation of α2u globulin and its mRNA (Roy et al., 1983). The multihormonal control of α2u globulin thus serves as a useful model for the study of hormonal interactions at both the systemic and cellular levels in the regulation of specific gene expression.

A body of clinical and experimental results suggest physiological interaction between thyroid hormone and insulin in the regulation of target cell function. Thus hypoinsulinemic diabetes, both in humans and in experimental animals, is frequently associated with an impaired thyroid function. This impairment is believed to be mediated through a decreased availability of the thyrotropin release factor (TRH) and a reduction in the extra thyroidal deiodination of T_4 (Gonzalez et al., 1980; Chopra et al., 1981; Wiersinga et al., 1982). In order to understand the biochemical basis of the interaction between insulin and thyroid hormone, we have examined the role of thyroxine in the reversal of insulin deficiency with respect to the synthesis of α2u globulin and its mRNA in the diabetic rat.

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