PHOTOCHEMICAL CROSSLINKING OF ATP TO ASPARTATE TRANSCARBAMYLASE

RUSSELL FRANCIS

CHEM 499

MAY 19, 1978

ABSTRACT

Evidence suggests that a covalent crosslink will occur between aspartate transcarbamylase (ATCase) and the adenine moiety of ATP upon irradiation of the complex by ultraviolet light of $\lambda > 290$nm in the presence of acetone. It is further suggested that the crosslinking is very specific to ATP and binds at the ATP receptor site. The C(8) of the adenisine ring is believed to be the site of attachment on the nucleotide. The use of acetone as a photosensitizer provides milder conditions (longer wavelengths) for this reaction to take place. This covalent crosslinking could provide the opportunity to identify the interacting regions of ATP to ATCase and more importantly the mechanism that ATP allosterically effects ATCase.

INTRODUCTION

Aspartate transcarbamylase catalyzes the first reaction unique to the biosynthesis of pyrimidine nucleotides. The ATCase molecule (molecular weight, 310,000) consists of two catalytic and three regulatory, or allosteric subunits. CTP is an allosteric inhibitor of ATCase. ATP is an allosteric activator. They bind on

the regulatory subunits, but it is not cleary understood whether they bind at the same sites. It appears that ATP is bound at 5.1 to 6 sites with similar affinities (Gray et al,1972). Although the sequence of the regulatory subunit is known, the locations of ATP and CTP binding are not yet known. The regulatory subunit consists of 152 residues (Weber,1968).

DISCUSSION

In acetone photosensitized reactions the substrate undergoing the chemical reactions is not that which absorbs the incident light but is the one which is excited through energy transfer from acetone (Elad,1976). Thus in the photolysis of purines, ultraviolet (UV) light may be absorbed solely by the acetone molecules. Peroxides and quinones have also been successfully used as photosensitizers (Elad,1976;Elad,Rosenthal,and Sasson,1971). Higher yields were observed when initiating the reactions with acetone than with direct 254nm light (Sperling,1976). Sperling successfully crosslinked ATP to histone H4,using acetone as the photosensitizer,with a 48% yield of crosslinked product. Protein precipitation and aggregation occured as a result of direct irradiation with 254nm light. 80% of his product proved to be monomeric.

This is not the case with pyrimidine nucleotides as photosensitized reactions of pyrimidines tend to enhance dimerization (Elad,Rosenthal,and Sasson,1971). An attempt to directly crosslink ATP to aminoacyl-tRNA synthetases (Yue and Schimmel,1977) using 254nm light provided only a 15% yield of crosslinked product.

Purine nucleosides undergo substitution at C(8) when irradiated in the presence of alcohols (Ben-Ishai et al,1973; Steinmaus,1969). In both the direct and photosensitized reations, the resulting substituent is the α-hydroxyalkyl group (Steinmaus et al,1971). These products have been isolated and their structures determined.

The initiation step involves the excitation of the purine by UV light followed by hydrogen removal from the alcohol by the excited purine (Elad,1976). This step results in the fragmentation of the alcohol to free radicals of the type R_1R_2COH which subsequently attack at the carbon end of imino-C=N groups in ground state purine (Fig.1). In photosensitized reactions there are two possible mechanisms that have been postulated (Sperling,1976; Elad,1976; Soloman and Elad,1974), 1)Acetone absorbs the incident light and transfers its excited energy to the purine which subsequently abstracts a hydrogen from the alcohol or, 2) The excited acetone molecule

abstracts the H atom from the alcohol leaving the alcohol free radical vulnerable to purine attack.

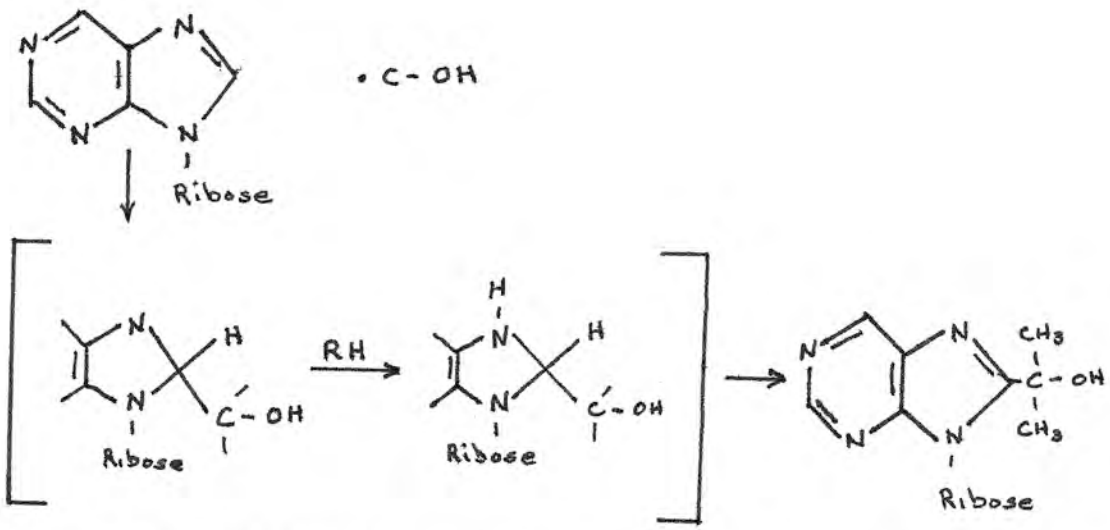


Figure 1: Postulated mechanism for alcohol substitution at C(8) of a purine nucleoside.

Studies on the effects of ionizing radiation of aqueous solutions of purine nucleosides and nucleotides were shown to involve opening of the imidizole ring (Elad,1976). Hems (1960) proposed that initial attack on the nucleoside by H or OH radicals leads to a nucleoside radical which recombines with a second radical. These reactions result in the introduction of a hydroxyl group at C(8) and an H atom at N(9) and leads to the opening of the C(8)-N(9) bond. The ribose is hydrolytically released.

Irradiation of caffeine, similar in structure to purines, in the presence of amines (Fig.2) leads to the substitution of an α- aminoalkyl or alkyl group for the hydrogen atom at C(8) (Solomon and Elad,1974).

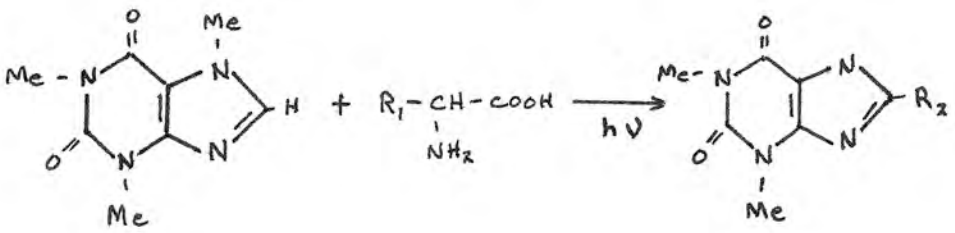
Figure 2: R= α-aminoalkyl or alkyl

Example:

Amine=isopropyl amine $R=(CH_3)_2CNH_2$

The photochemical reactions of caffeine with tetrahydrofuran, dioxane and other ethers have been studied (Elad,1976). The reactions resulted in substitution of an ether or alkyl group at C(8).

The photochemical reactions of caffeine with amino acids leads to the substitution of amino acids at C(8) (Elad and Rosenthal,1969). The nature of the product at C(8) depends upon the amino acid, but an alkyl group is present (Fig.3).



R_2=alkyl

Figure 3: Reaction of caffeine with amino acids.

Example:

R_1=alanine	R_2=Et
R_1=serine	R_2=Et
R_1=leucine	R_2=$Me_2CH(CH_2)_2$

Phosphorylation of sugar hydroxyls are not expected to influence the absorption of nucleosides, since the phosphates are insulated by the saturated ribose (Elad,1976). This is further verified by the fact that the absoption spectrum of nucleosides and nucleotides resemble one another.

In a recent study by Yue and Schimmel (1977) on the photochemical crosslinking of ATP to an aminoacyl-tRNA synthetase, they showed that the ATP receptor site is specific to ATP. This was done by attempting to crosslink the enzyme with AMP. They also attempted to crosslink ATP to serum albumin. In both cases no

crosslinking occured. The same results were obtained with no irradiation of an ATP-enzyme complex. They also determined that the crosslinking of ATP is proportional to the percent activation of the enzyme (Table 1).

Table 1: Effect of Preirradiation on Crosslinking Yields

Effective preirradiation dose ($nEinsteins/mm^2$)	%act.	% of maximal crosslinking
0	100	100
1.7	91	89
26	21	23
51	15	15
102	5	≤14

The enzyme was deactivated by preirradiation for varying times and tested for crosslinking yield. The dose of irradiation in their experiment increased ATP crosslinking in a monotonic fasion until it reached a saturated level of about 0.15 mole of ATP per mole of synthetase. Any further irradiation did not increase the crosslinking yield. Their maximum effective dose

was approximately 15-20nE/mm^2. In an attempt to determine whether the whole ATP molecule was incorporated they labeled different parts of the purine nucleotide and crosslinked them (Table 2). The same yield was produced in each case, therefor it appears that the whole nucleotide is incorporated.

Table 2: Photocrosslinking Yields for Ile-tRNA Synthetase Labeled ATP Mixtures.

Compound	Moles of ATP joined/ mole of Ile-tRNA synthetase
(U-^{14}C)ATP	0.142
(α-^{32}P)ATP	0.163
(γ-^{32}P)ATP	0.148

They made an attempt to identify the crosslinked residue but the sequence of the synthetase is not known. It was established that the acid hydrolysis procedures used in the Dansyl-Edman method of protease digestion were harsh enough to rupture the linkage between the nucleotide and the residue. But using (α-^{32}P)ATP they did eludicate a labeled peptide that consisted of Lys-Val-Ala-Gly-Asx-X. The X does not correspond to any amino acid. X could be the site of ATP attachment.

To irradiate their samples, Yue and Schimmel used 20-25nmole of ATP per 1nmole of enzyme. This mixture was placed as a droplet on Parafilm on ice and irradiated with a 15-watt low pressure mercury lamp from 4cm for 30-40 minutes. Their lamp irradiated 253.7nm light.

Sperling (1976) crosslinked ATP to histone H4 using acetone as a sensitizer. He showed that a covalent bond formed between ATP and histone H4. This was demonstrated by incorporation of radioactive nucleotide into the protein, and showed that the radioactivity remained associated with the protein fractions in high voltage electrophoresis, ion exchange chromatography, and gel filtration. He noted that irradiation of ATP by itself did not alter the nucleotide any. Sperling's method of irradiation, was to irradiate a nucleotide to protein molar ratio of 0.9 to 1.0, by a 200-watt super pressure mercury lamp, 15cm from the lamp in a 0.5cm quartz cell. A Pyrex filter was used to obtain a light of $\lambda > 290$nm. The sample was constantly maintained at 25 degrees. The amount of acetone used was not discussed.

CONCLUSION

The advantages of using near UV light, which is not absorbed by either partener, and acetone to mediate crosslinking are: 1) excessive distruction of light sensitive amino acids is avoided and 2) protein to protein crosslinking is minimized (Sperling, 1976).

By analogy to the reactions of purines and caffeine with alcohols, ethers, amines, and amino acids, it seems likely that the C(8) of the adenosine ring would be the location of the protein-nucleotide bond.

Once the proper ratios of ATP, ATCase, and acetone are found and the proper amount of irradiation is determined, sucessfull crosslinking yields should be obtained. Since the sequence of the ATCase regulatory chain is known, protease digestion should eludicate identifiable peptides from which the mechanism of photochemical bonding may be postulated. The number of ATP linked peptides may answer the question as to how many ATP binding sites are available on the regulatory chain. This information in turn would provide a better understanding of the mechanism of allosteric enzymes.

REFERENCES

Ben-Ishai,R.,M.Green,E.Graff,D.Elad,H.Steinmaus and J.Soloman, (1973), Photochem. Photobiol. (17) 155-167

Elad,D., Photochemistry and Photobiology of Nucleic Acids, Vol.1, Editor: Wang,S., Academic Press, New York, (1976), 357-380

Elad,D., and I.Rosenthal, (1969), Chem. Commun. 905-906

Elad,D., I. Rosenthal, and S. Sasson, (1970) J. Chem Soc.,(C), 2053-2057

Gray,W., M.Chamberlin, and D. Gray, (1973) J. Biol. Chem. (248), 6071-6079

Hems,G., (1960), Radiat. Res. (13), 777

Soloman,J., and D.Elad, (1974), Photochem. Photobiol., (19), 21-28

Sperling,J., (1976), Photochem. Photobiol.,(23), 323-326

Steinmaus,H.,I.Rosenthal, and D.Elad, (1969), J. Amer. Chem. Soc., (91), 4921-4923

Steinmaus,H.,I.Rosenthal, and D.Elad, (1971), J. Org. Chem., (36), 3594

Weber,K., (1968), Nature, (218), 1116-1119

Yue,V., and P.Schimmel, (1977), Biochemistry, (16) 4678-4683