Evidence for the Conformation of Enzyme-Bound Adenosine 5′-Phosphate. Substrate and Inhibitor Properties of 8,5′-Cycloadenosine 5′-Phosphate with Adenylate Kinase, Adenylate Aminohydrolase, Adenylosuccinate Lyase, and 5′-Nucleotidase†

Alexander Hampton,* Peter J. Harper, and Takuma Sasaki

ABSTRACT: Phosphorylation of 2′,3′-*O*-isopropylidene-8,5′-cycloadenosine followed by removal of the isopropylidene group has furnished 8,5′-cycloadenosine 5′-phosphate (8,5′-cyclo-AMP) as a 1:3 ratio of 5′ epimers of which only the minor one was a substrate of venom 5′-nucleotidase and pig muscle AMP kinase. With AMP aminohydrolase of rabbit muscle the epimeric mixture gave essentially the same V_{max} as AMP and a K_m of 2.0 mM (AMP, V_{max} = 1140 μmol/min per mg of protein; K_m = 0.3 mM). With snake venom 5′-nucleotidase, V_{max} of the active epimer (0.5–0.75 μmol/min per mg of protein) was comparable to that of AMP (1.25 μmol/min per mg of protein). With AMP kinase, 8,5′-cyclo-AMP reacted twice as rapidly as the same level (12.5 μM) of AMP. The 8,5′-cyclo-ADP so produced was a substrate of rabbit muscle pyruvate kinase. These data together with evidence that the phosphate moiety, the furanose ring, and the adenine ring of AMP all are required for or promote catalytic efficiency, indicate that enzyme-bound AMP probably possesses a sugar–base torsion angle similar to that of 8,5′-cyclo-AMP and that the 5′-oxygen of AMP is most likely oriented in a direction between H-3′ and H-4′. 8,5′-Cyclo-AMP was a strong competitive inhibitor of AMP aminohydrolase (apparent K_i = 55 μM; K_m (for AMP) = 0.3 mM) and of the conversion of adenylosuccinate to AMP by *Escherichia coli* adenylosuccinate lyase (K_i = 8 μM; K_i (for AMP) = 13 μM), suggesting that in aqueous solution the conformation of AMP may resemble that postulated for enzyme-bound AMP. The findings show that catalytic conversion of AMP by the above three enzymes is unlikely to be accompanied by significant rotation about the 4′,5′ and glycosidic bonds or puckering of the ribofuranose ring of AMP.

Adenosine 5′-phosphate (AMP) (Figure 1) has the potential of existing under physiological conditions as one or more of a large number of conformers which could arise from modes of puckering of its furanose ring and from rotation about the bonds between N-9 and C-1′, C-4′ and C-5′, C-5′ and O-5′, C-2′ and O-2′, C-3′ and O-3′, and C-6 and N-6. The most extensive conformational changes in AMP are associated with ribofuranose puckering and with rotation about the 9,1′ and 4′,5′ bonds. Proton magnetic resonance (pmr) studies (Schweizer *et al.*, 1968; Danyluk and Hruska, 1968; Feldman and Agarwal, 1968) have revealed that in aqueous solution the phosphate group of AMP is closer to H-8 than to H-2 and hence that AMP exists principally as one or more anti conformers (illustrated in Figure 1) in which H-8 is situated over the ribofuranose ring. Proximity of H-8 to the phosphate group could occur in a range of AMP conformers differing from each other in sugar–base torsion angle by as much as 120°. Analysis of lanthanide ion shift and broadening of pmr signals of AMP (Barry *et al.*, 1971) has led to more precise delineation of the conformation of AMP in aqueous solution at pH 2, but similar studies at physiological pH have not yet been reported. The present communication presents evidence for the conformation in aqueous solution (pH 6.5–8.5) of free and enzyme-bound AMP which derives from studies of the interaction of an AMP derivative of fixed conformation with AMP-utilizing enzymes. We recently synthesized a derivative of 2′,3′-*O*-isopropylideneadenosine (II, Figure 2) which is constrained in a pronounced anti position by a bond between C-8 and C-5′ (Harper and Hampton, 1972), and we now report the chemical conversion of this protected nucleoside to 8,5′-cyclo-AMP (IV).[1] This nucleotide has previously been obtained as a product of the γ radiolysis of AMP in aqueous solution (Keck, 1968). Substrate and inhibitor properties of this extensively rigidified analog of AMP with four enzymes are described and are concluded to have direct implications regarding the conformation of AMP prior to and during its enzyme-catalyzed transformations.

Materials and Methods

Thin-layer chromatography (tlc) was carried out on cellulose in the following systems: (A) 1-butanol–acetic acid–water (4:1:5), (B) isopropyl alcohol–ammonia–water (7:1:2), and (C) ethanol–1 M ammonium acetate (7:3).

Preparation of 8,5′-Cycloadenosine 5′-Phosphate. 2′,3′-*O*-Isopropylidene-8,5′-cycloadenosine (Harper and Hampton, 1972) (90 mg) was phosphorylated by the general method of Yoshikawa and coworkers (1969) for the conversion of isopropylidene nucleosides to nucleoside 5′-phosphates. Thin-

† From the Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania 19111. *Received June 5, 1972.* This work was supported by U. S. Public Health Service Grant CA-11196, American Cancer Society Grant T-490, an award from the Pennsylvania Science and Engineering Fund, and by grants to The Institute for Cancer Research (U. S. Public Health Service Grants CA-06927 and RR-05539 and an appropriation from the Commonwealth of Pennsylvania).

[1] Abbreviations used are: 8,5′-cyclo-AMP, 8,5′-cycloadenosine 5′-phosphate. Other abbreviations are given in *Biochemistry 5*, 1445 (1966).

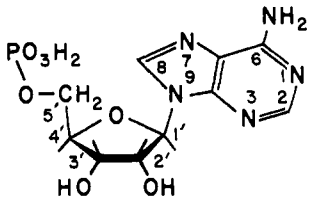

FIGURE 1: Anti conformation of AMP. The position of O-5′ in relation to the ribofuranose ring was selected arbitrarily. The trans relationship of the phosphoryl group to C-4′, which is shown here, has been indicated by proton magnetic resonance data (for references, see text) and by ^{31}P magnetic resonance studies (Tsuboi *et al.*, 1968).

layer chromatography showed 10% conversion had occurred after 8 hr at 25° with 10 molar equiv of $POCl_3$ and that additional conversion did not occur with longer reaction times. The reaction mixture was treated with diethyl ether (150 ml) after 10 hr and the precipitate so obtained was washed by decantation and treated with water (5 ml) and ice (*ca.* 5 g) with stirring for 30 min. The mixture was adjusted to pH 2.2 with $Ba(OH)_2$ and held at 90° (internal) for 30 min. The mixture was adjusted to pH 8.0 with $Ba(OH)_2$ and allowed to stand overnight at 4°. The precipitated barium salts were removed by centrifugation and the supernatant was treated with ethanol (30 ml) and the insoluble barium nucleotides were collected by centrifugation. Thin-layer chromatography showed the presence in this product of four ultraviolet-absorbing components. Spray tests showed that the two components of higher R_F contained no phosphorus and only one of the remaining two contained a *cis*-glycol system. The four components were readily separated upon chromatography of the product on Dowex 1 (Cl^-) (2.5 ml of wet resin) with 50 ml of a gradient of 0.001–0.005 N HCl. Fractions containing the desired component were neutralized (pH 8) with $Ba(OH)_2$ and two volumes of ethanol added to the clear solution. The barium nucleotide so obtained was dissolved in water, passed over potassium Dowex 50 (2.5 ml of wet resin) and the column washed until uv-absorbing material was removed. The combined washings were lyophilized to give 3.9 mg of a hygroscopic white powder. This material was homogeneous by thin-layer chromatography in systems A, B, and C (R_F 0.20, 0.40, and 0.22, respectively, compared to 0.15, 0.38, and 0.28 for AMP). Upon electrophoresis in 0.05 M ammonium formate (pH 8.0) the product had a mobility of 17.5 cm compared to 17.4 cm for AMP. Spectrophotometric determination with periodate of the *cis*-glycol moiety (Dixon and Lipkin, 1954) gave a ratio to the base of 0.94:1.00 and the rate of uptake of periodate was essentially the same as in the case of AMP. The uv spectra showed λ_{max} 263 nm at pH 2 (ϵ 14,800) and λ_{max} 267 nm at pH 11 (ϵ 14,000) and were identical with those reported for fully characterized 8,5′-cyclo-AMP obtained by γ radiolysis of AMP (Keck, 1968), and as expected, very similar to the values for 2′,3′-*O*-isopropylidene-8,5′-cycloadenosine (previously cited incorrectly; Harper and Hampton, 1972): pH 1, 262 nm (ϵ 14,500); pH 11, 267 nm (ϵ 13,900). Alkaline phosphatase suspension (Boehringer, 0.01 ml) was added to a solution of the product (0.4 mg) in 0.1 ml of 0.1 M glycine buffer–0.001 M $MgCl_2$ (pH 10). After 3 hr at 37° complete conversion occurred to a product which was chromatographically indistinguishable from 8,5′-cycloadenosine (Hampton *et al.*, 1973) in solvent systems A, B, and C.

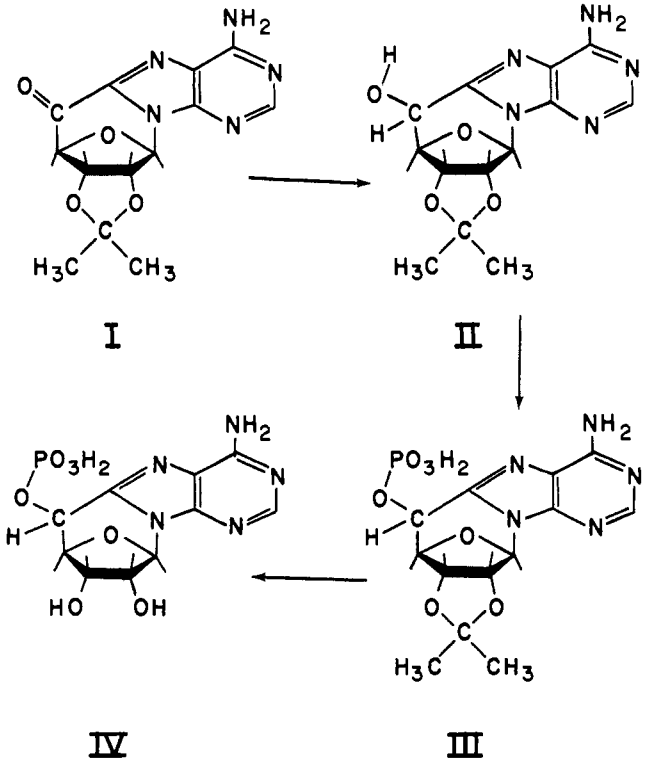

FIGURE 2: Synthesis of 8,5′-cyclo-AMP.

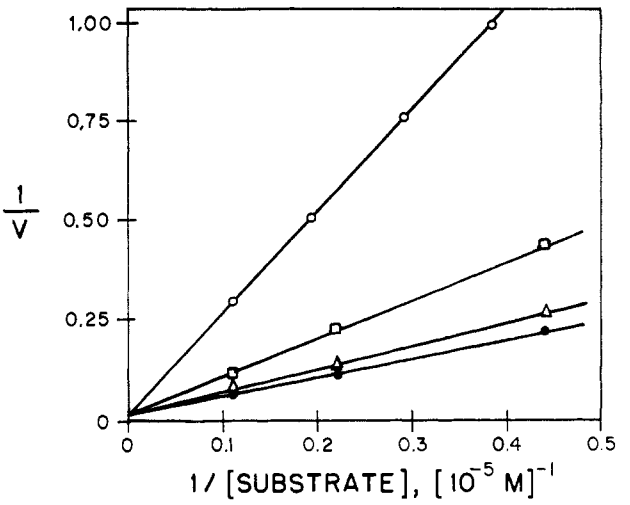

FIGURE 3: 8,5′-Cyclo-AMP as substrate and inhibitor of AMP deaminase. 8,5′-Cyclo-AMP alone (○), AMP alone (●), AMP and 26 μM 8,5′-cyclo-AMP (△), AMP and 53 μM 8,5′-cyclo-AMP (□). Velocity is expressed as nmol of substrate converted per min.

Chemicals for Enzyme Assays. AMP, ATP, and NADH were from P-L Biochemicals, Inc., and phosphoenolpyruvate was from Sigma Chemical Co.

Enzyme Kinetic Studies. All assays were carried out by measurement of the rate of change of optical density (OD) at a suitable wavelength in a Cary Model 15 spectrophotometer using 1-cm cells containing a final volume of 0.95 ml. In all systems the initial velocity with AMP as substrate was linear and proportional to the concentration of primary enzyme and independent of the concentration of secondary enzymes used in coupled assays. In the case of the AMP aminohydrolase and 5′-nucleotidase assay systems the primary enzyme was also rate limiting with 8,5′-cyclo-AMP as substrate.

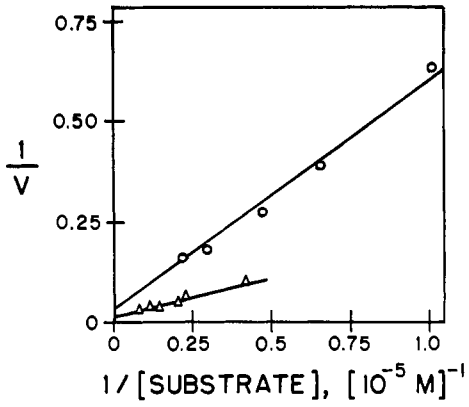

FIGURE 4: Dephosphorylation of AMP (Δ) and 8,5′-cyclo-AMP (○) by 5′-nucleotidase. Velocity is in nmol × 10^{-1} of substrate converted per min.

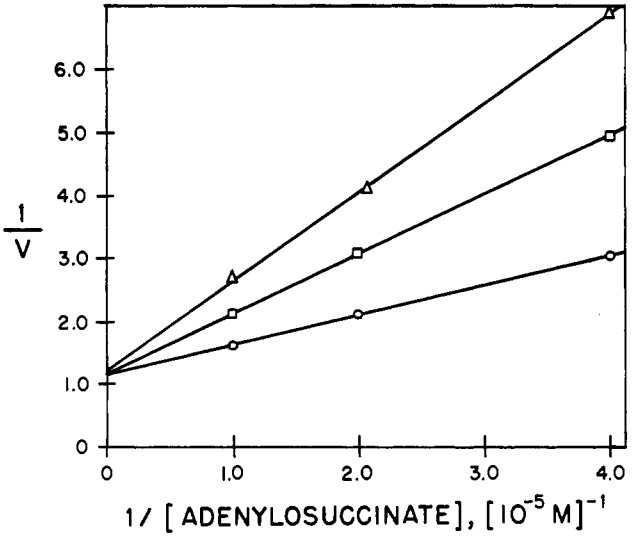

FIGURE 5: Cleavage of adenylosuccinate by adenylosuccinate lyase: no inhibitor (○); 5 μM 8,5′-cyclo-AMP (□); 10 μM 8,5′-cyclo-AMP (Δ). Velocity is in μmol per min.

AMP aminohydrolase (Sigma, grade IV, rabbit muscle, 0.057 μg) was employed in a system 0.01 M in citrate and 0.005 M in KCl (pH 6.5). The decrease in OD was measured at 265 nm where Δε for the conversion was 6600.

5′-Nucleotidase (Sigma, grade II, from *Crotalus adamanteus* venom, 4 μg) was used in a coupled system with adenosine deaminase (Sigma, type I, from intestinal mucosa, 100 μg) in 0.1 M Tris-HCl (pH 8.5). Measurement and calculation of Δε were identical with AMP aminohydrolase.

AMP kinase (Boehringer, pig muscle, 1.0 μg) was studied in a system 0.1 M in Tris HCl, 0.001 M in $MgSO_4$ and 0.13 M in KCl (pH 7.6) containing 125 μg of P-enolpyruvate (cyclohexylammonium salt), 500 μg of ATP (sodium salt), 125 μg of NADH (sodium salt), 250 μg of pyruvate kinase (Boehringer), and 250 μg of lactic dehydrogenase (Boehringer). The OD change was measured at 340 nm.

Partially purified adenylosuccinate lyase of *E. coli* was studied in 20 mM Tris-HCl–20 mM NaEDTA buffer at pH 7.8; this reaction medium was employed in kinetic studies of adenylosuccinate lyase of yeast (Bridger and Cohen, 1968). The OD change of the cleavage reaction was measured at 280 nm.

Results

8,5′-Cyclo-AMP as a Substrate and Inhibitor of AMP Aminohydrolase. Figure 3 shows that the 8,5′-cyclo-AMP was a substrate and gave a linear reciprocal rate plot. The maximal velocity (V_{max}) was 1140 μmol/min per mg of protein and the Michaelis constant (K_m) was 2.0 mM; with AMP as substrate (Figure 3), V_{max} was 1140 μmol/min per mg of protein and K_m was 0.3 mM under the same conditions. The values for AMP are similar to those calculated from the results of Smiley *et al.* (1967) (V_{max} = 1380 μmol/min per mg of protein; K_m = 0.5 mM).

The 8,5′-cyclo-AMP was a competitive inhibitor of the deamination of AMP (Figure 3). The results tend to suggest that the inhibition might be nonlinear. A secondary plot from Figure 3 of slope against the square of the inhibitor concentration was linear and gave an apparent root-mean-square enzyme–inhibitor dissociation constant of 55 μM.

8,5′-Cyclo-AMP as a Substrate of 5′-Nucleotidase. With AMP as substrate (Figure 4), K_m was 0.13 mM and V_{max} was 1.25 μmol/min per mg of protein. An enzyme preparation from the same commercial source was reported by Murray and Atkinson (1968) to have V_{max} = 1.39 μmol/min per mg of protein and K_m = 0.035 mM. 8,5′-Cyclo-AMP showed pronounced but less substrate activity than AMP at all concentrations tested with a V_{max} in the range of 0.5–0.75 μmol/min per mg of protein. If the reactions were allowed to go to completion only 22% of the 8,5′-cyclo-AMP underwent reaction. The 5′ epimers of 8,5′-cycloadenosine have been chemically synthesized (Hampton *et al.*, 1973) and it was found that a mixture of the two was rapidly and completely deaminated by the amount of adenosine deaminase employed in the above coupled assay of 5′-nucleotidase. This establishes that only one of the two epimers of 8,5′-cyclo-AMP is a substrate for 5′-nucleotidase and that this epimer constitutes 22% of the total.

Inhibition of Adenylosuccinate Lyase by 8,5′-Cyclo-AMP. 8,5′-Cyclo-AMP inhibited formation of AMP competitively with respect to adenylosuccinate (Figure 5). A replot of slope against inhibitor concentration was linear and gave a K_i value of 8.0 μM. AMP was similarly studied with the same enzyme preparation and also gave linear competitive inhibition and a K_i of 13 μM. AMP inhibits highly purified yeast adenylosuccinate lyase in the same manner and with a similar effectiveness (K_i = 12 μM) (Bridger and Cohen, 1968). That AMP underwent no change during these studies was demonstrated by treating it with the *E. coli* lyase for double the period required for the initial velocity measurements, after which AMP aminohydrolase was added, when the rate and extent of absorbance decrease at 265 nm showed that no deamination or dephosphorylation of the AMP had occurred; adenosine is deaminated by this AMP aminohydrolase only 1% as rapidly as is AMP itself (Zielke and Suelter, 1971).

8,5′-Cyclo-AMP as a Substrate of Adenylate Kinase. With AMP as substrate, K_m was 150 μM and V_{max} was 93 μmol/min per mg of protein. With 50 μM 8,5′-cyclo-AMP, an initial velocity of 10 nmol/min was obtained in duplicate experiments compared to an initial velocity of 16 nmol/min for AMP at the same level. These reactions were allowed to go to completion (15 min) and it was calculated (see Discussion) from the absorbance change at 340 nm that 27% of the cyclo-AMP and all of the AMP had reacted. No additional change in absorbance at 340 nm occurred during a further hour. From the K_m value of AMP it was calculated that at the actual con-

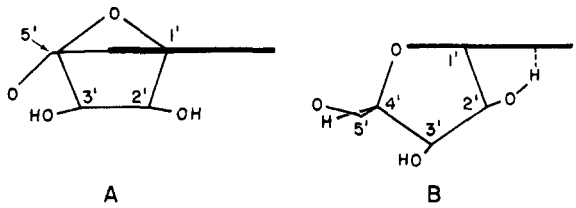

FIGURE 6: Partial structures of 8,5′-cyclo-AMP ((A); the postulated enzyme-bound 5′ epimer is shown) and AMP with a hydrogen bond between O-2′ and N-3 (B). The adenine ring is shown in edge-on view as a thickened line; the phosphoryl groups and carbon-bound hydrogens (except H-4′ in B) have been omitted.

centration tested (12.5 μM) the active epimer of 8,5′-cyclo-AMP underwent reaction almost twice as rapidly as would the same concentration of AMP.

Discussion

Conversion of I to II creates a new asymmetric center at C-5′. Data from the enzyme studies, discussed below, show that the ratio of the 5′ epimers of 8,5′-cyclo-AMP is approximately 3:1. The unequal amounts of epimers might have arisen as a result of stereoselectivity during the reduction of I to II, because reduction of bicyclic ketones by lithium aluminum hydride (Brown and Deck, 1965) and of monocyclic ketones by sodium borohydride (House *et al.*, 1962) are known to occur stereoselectively. That the nucleoside II was a mixture of epimers was suggested by its pmr spectrum (Harper and Hampton, 1972); unsuccessful attempts were made to separate these epimers by silica gel chromatographic procedures similar to those which enable separation of the stereoisomeric 5′-*C*-methyl-2′,3′-*O*-isopropylideneadenosines (Howgate and Hampton, 1972).

Snake venom 5′-nucleotidase dephosphorylated only 22% of the 8,5′-cyclo-AMP, and Figure 4 shows that the V_{max} of the active epimer is similar in magnitude to that of AMP itself. The mixed epimers of 8,5′-cyclo-AMP showed substrate activity also with AMP aminohydrolase of rabbit muscle (Figure 3) and in this case the V_{max} was essentially the same as that of AMP. When 8,5′-cyclo-AMP was tested as a substrate for pig muscle AMP kinase in a coupled assay system with pyruvate kinase and lactate dehydrogenase, 27% of the maximum amount of NAD was produced. The initial velocity was almost twice that with the same level of AMP as substrate. If solely the epimer which constituted *ca.* 75% of 8,5′-cyclo-AMP were a substrate of AMP kinase, either 75 or 37.5% of the maximum amount of NAD would be formed, depending upon whether the stereochemically corresponding 8,5′-cyclo-ADP was or was not a substrate of pyruvate kinase under the assay conditions. The result obtained shows unequivocally that only the minor epimer, amounting to 27% of the 8,5′-cyclo-AMP, is a substrate of AMP kinase, and that the 8,5′-cyclo-ADP thereby produced is likewise a substrate of rabbit muscle pyruvate kinase. The value of 27% for the proportion of minor epimer agrees within experimental error with the value of 22% given by 5′-nucleotidase.

The ribose carbons of 8,5′-cyclo-AMP together with C-8 and N-9 comprise a seven-membered ring bridged by the furanose oxygen. The rigidity associated with a bridged-ring system is reinforced in this case by the resistance to rotation about the 8,9 bond imposed by the fused imidazole ring. In consequence, the 1′, 4′, 5′, 8, and 9 atoms are essentially coplanar with the purine ring system and the ribofuranose ring is considerably more rigid than the ribofuranose ring of AMP. The present findings indicate that one of the two C-5′ epimers of 8,5′-cyclo-AMP is comparable to AMP in its rate of catalytic transformation by AMP aminohydrolase, AMP kinase, and 5′-nucleotidase. In view of the rigidity of 8,5′-cyclo-AMP, this result implies that the catalytic processes of all three enzymes can proceed efficiently without rotation of the 9,1′ or 4′,5′ bonds and without changes in conformation of the ribofuranose ring of AMP.

The nature of the catalyzed reactions, together with certain of the substrate specificity properties (listed below) of the foregoing three enzymes, are consistent with the view that these enzymes interact during catalysis with all three areas of AMP, namely, with the purine ring, the ribofuranose ring, and the phosphate moiety. Thus it seems reasonable to assume that the phosphate portion of AMP interacts with AMP kinase and 5′-nucleotidase and that the adenine portion interacts with AMP aminohydrolase. That the remaining principal segments of AMP interact with these enzymes is indicated by the substrate properties of compounds derived from AMP by substitution of a hydrogen atom for a group (*e.g.*, phosphoryl or hydroxyl) which has potential for interaction with enzymic groups. Thus for rabbit muscle AMP aminohydrolase, maximum velocity data show that 2′-dAMP and adenosine are deaminated 18 and 1%, respectively, as rapidly as AMP (Zielke and Suelter, 1971); in addition, 3′-dAMP is deaminated at a rate approximately 0.1% that of AMP (A. Hampton and T. Sasaki, 1972, unpublished data). Snake venom 5′-nucleotidase of *Crotalus adamanteus* attacks ribose 5-phosphate 2% as rapidly as AMP (Heppel and Hilmoe, 1951) and attacks 2′- and 3′-dAMP at 16 and 11%, respectively, the rate of AMP (A. Hampton and T. Sasaki, 1972, unpublished data). Likewise, the venom 5′-nucleotidase of *Bothrops atrox* does not attack ribose 5-phosphate and cleaves 2′-dAMP 39% as rapidly as AMP (Sulkowski *et al.*, 1963); the venom enzyme from *Trimeresurus flavoviridis* is unable to attack the AMP analog 9-(4′-hydroxybutyl)-6-aminopurine 4′-phosphate (Mizuno *et al.*, 1961). Finally, AMP kinase of pig muscle exhibits considerable specificity for the adenine portion of AMP (neither IMP nor GMP are substrates; Dr. Mildred Cohn, personal communication), and it acts upon 2′-d- or 3′-dAMP only *ca.* one-third as rapidly as on AMP (A. Hampton and T. Sasaki, 1972, unpublished data). The 2′- and 3′-hydroxyls of AMP may partly or wholly influence V_{max} values by affecting the conformation of enzyme-bound AMP *via* an intramolecular action rather than by interacting with enzymic groups. Thus the 2′-OH may form a hydrogen bond with N-3, as suggested by certain pmr data (Schweizer *et al.*, 1968) while O-3′ is capable of influencing the spatial position of O-5′ by exerting a repulsive force on it.

Interaction of the enzymic catalytic sites with the phosphate, ribose, and adenine segments of AMP would, presumably, be simultaneous at some stage of the catalytic process. This simultaneous interaction would have the effect of curtailing rotation about the glycosidic and 4′,5′ bonds to within narrow confines set by the flexibility of the AMP catalytic site complex. That 8,5′-cyclo-AMP is incapable of rotation about the glycosidic or 4′,5′ bonds, and that one of its two 5′ epimers substitutes efficiently for AMP as a substrate suggests, on this view, that during the rate-determining events of the catalytic process the adenine ring of AMP approximates to the degree of anti conformation possessed by 8,5′-cyclo-AMP and that the favored 4′,5′ rotamer is one wherein O-5′ of AMP is situated to either one side or the other of the plane defined by the 1′, 4′, and 5′ carbons, as illustrated in the

structure IV of 8,5′-cyclo-AMP. It appears unlikely that O-5′ of enzyme-bound AMP would neighbor on O-4′ (as in the case of one of the 5′ epimers of 8,5′-cAMP), since this would be mitigated against by powerful oxygen–oxygen repulsion. Hence, O-5′ is more probably in a similar location to O-5′ of the other 5′ epimer of 8,5′-cyclo-AMP, *i.e.*, above and between H-3′ and H-4′, as illustrated in structure A (Figure 6). This conformation, moreover, is stabilized by staggered 4′ and 5′ substituents and by equidistance of O-5′ from O-4′ and O-3′.

As described above, the 2′- and 3′-hydroxyls of AMP do not necessarily promote catalysis *via* interaction directly with the enzymes, although this mechanism might well operate in the case of AMP aminohydrolase for which the 3′-OH makes an exceptionally large (1000-fold) contribution to V_{max}. In the event that the 2′-OH of enzyme-bound AMP were hydrogen bonded to N-3 (Figure 6, structure B) H-8 would be located immediately above O-4′. If, in addition, O-5′ were located above H-4′, then the adenine ring and phosphate group of the AMP would be spatially related to each other in almost identical manner as they are in that 5′ epimer of 8,5′-cyclo-AMP in which O-5′ is situated between H-3′ and H-4′ (Figure 6). On this basis, the present findings indicate that were an O-(2′)–N(3) hydrogen bond indeed present, then H-8 of enzyme-bound AMP would likely be situated at or between O-4′ and C-4′, and O-5′ would be near H-4′.

8,5′-cyclo-AMP was a linear competitive inhibitor of the conversion of adenylosuccinate to AMP by *E. coli* adenylosuccinate lyase (Figure 5), showing that it combines solely with uncomplexed enzyme. The enzyme–inhibitor dissociation constant (8 μM) was essentially the same as the value (13 μM) given by AMP itself with the same preparation of the enzyme. The cyclo-AMP also exhibited inhibition and substantial binding (apparent K_i = 55 μM; K_m (for AMP) = 300 μM) toward AMP aminohydrolase. Studies with yeast adenylosuccinate lyase indicate that the adenine ring and the phosphate moiety of AMP both participate in formation of the AMP–enzyme complex (Bridger and Cohen, 1968). The present evidence that 8,5′-cyclo-AMP can bind strongly to AMP sites therefore suggests that the adenine–ribose torsion angle and preferred 4′,5′ rotamer of AMP in aqueous solution may resemble those postulated above for enzyme-bound AMP. This conclusion is in harmony with extensive proton magnetic resonance evidence (Schweizer *et al.*, 1968; Danyluk and Hruska, 1968; Feldman and Agarwal, 1968; Barry *et al.*, 1971) that in aqueous solution the phosphate group of AMP is closer to H-8 than to H-2.

Acknowledgments

The authors thank Dr. L. H. Cohen for kindly providing adenylosuccinic acid and adenylosuccinate lyase of *E. coli*.

References

Barry, C. D., North, A. C. T., Glasel, J. A., Williams, R. J. P., and Xavier, A. V. (1971), *Nature (London) 232*, 236.

Bridger, W. A., and Cohen, L. H. (1968), *J. Biol. Chem. 243*, 644.

Brown, H. C., and Deck, H. R. (1965), *J. Amer. Chem. Soc. 87*, 5620.

Danyluk, S. S., and Hruska, F. E. (1968), *Biochemistry 7*, 1038.

Dixon, J. S., and Lipkin, D. (1954), *Anal. Chem. 26*, 1092.

Feldman, I., and Agarwal, R. P. (1968), *J. Amer. Chem. Soc. 90*, 7329.

Hampton, A., Harper, P. J., and Sasaki, T. (1973), *Biochemistry* (in press).

Harper, P. J., and Hampton, A. (1972), *J. Org. Chem. 37*, 795.

Heppel, L. A., and Hilmoe, R. J. (1951), *J. Biol. Chem. 188*, 665.

House, H. O., Babad, H., Toothill, R. B., and Noltes, A. W. (1962), *J. Org. Chem. 27*, 4141.

Howgate, P., and Hampton, A. (1972), *Carbohyd. Res. 21*, 309.

Keck, K. (1968), *Z. Naturforsch. 23 B*, 1034.

Mizuno, Y., Ikehara, M., Ueda, T., Nomura, A., Ohtsuka, E., Ishikawa, F., and Kanai, Y. (1961), *Chem. Pharm. Bull. 9*, 338.

Murray, A. W., and Atkinson, M. R. (1968), *Biochemistry 7*, 4023.

Schweizer, M. P., Broom, A. D., Ts'o, P. O. P., and Hollis, D. P. (1968), *J. Amer. Chem. Soc. 90*, 1042.

Smiley, K. L., Barry, A. J., and Suelter, C. H. (1967), *J. Biol. Chem. 242*, 2502.

Sulkowski, E., Bjork, W., and Laskowski, M. (1963), *J. Biol. Chem. 238*, 2477.

Tsuboi, M., Kainosho, M., and Nakamura, A. (1968), *in* Recent Developments of Magnetic Resonance in Biological Systems, Fujiwara, S., and Piette, L. H., Ed., Tokyo, Hirokawa Publishing Co., p 43.

Yoshikawa, M., Kato, T., and Takenishi, T. (1969), *Bull. Chem. Soc. Jap. 42*, 3505.

Zielke, C. L., and Suelter, C. H. (1971), *J. Biol. Chem. 246*, 1313.