Studies on the Kinetic Mechanism of *S*-Adenosylmethionine:Protein *O*-Methyltransferase of Calf Thymus†

M. Jamaluddin, S. Kim, and W. K. Paik*

ABSTRACT: Initial velocity studies have been carried out on protein methylase II (*S*-adenosyl-L-methionine:protein *O*-methyltransferase, EC 2.1.1.24) purified from calf thymus, using bovine pancreatic ribonuclease as the protein substrate. Initial velocity patterns converging at a point on or near the extended abcissa were obtained with either ribonuclease or *S*-adenosyl-L-methionine as the variable substrate. Inhibition by the product *S*-adenosyl-L-homocysteine was linear competitive against both *S*-adenosyl-L-methionine and ribonuclease, the apparent inhibition constants being dependent on the concentration of the nonvaried substrate. Adenosine was an inhibitor of the reaction, the inhibition being linear competitive against both *S*-adenosyl-L-methionine (K_i / 1.2×10^{-3} mol/l.) and ribonuclease (K_i / 4.6×10^{-3} mol/l.). These results are consistent with a random mechanism for the protein methylase II reaction in which the rate-limiting step may be the interconversion of the ternary complexes and all other steps may be in equilibrium. The limiting Michaelis constants for *S*-adenosyl-L-methionine and ribonuclease are 0.87×10^{-6} and 2.86×10^{-4} mol/l., respectively. The dissociation constants of *S*-adenosyl-L-methionine and ribonuclease for their reaction with the free enzyme are 0.97×10^{-6} and 3.35×10^{-4} mol/l., respectively. The dissociation constant of *S*-adenosyl-L-homocysteine for its reaction with the free enzyme was 1.03×10^{-6} mol/l. Thus it has about equal affinity for calf thymus protein methylase II as *S*-adenosyl-L-methionine.

Several enzyme systems which catalyze the transfer of the methyl group of *S*-adenosyl-L-methionine to diverse biological molecules of varying complexity are now known (Greenberg, 1963; Paik and Kim, 1971; Kerr and Borek, 1973). A number of specific methyltransferases are involved in the methylation of proteins (Paik and Kim, 1971) and nucleic acids (Kerr and Borek, 1973). Recently protein methylase II (*S*-adenosylmethionine:protein *O*-methyltransferase, EC 2.1.1.24) from calf thymus has been purified to apparent homogeneity in our laboratory (Kim, 1973). The enzyme catalyzes the methyl esterification of free carboxyl groups of side chains of a protein substrate (Kim and Paik, 1971a). It shows a broad specificity with regard to the protein substrate. Bovine pancreatic ribonuclease, histones F_1, F_2, and F_3, γ-globulin, and ovalbumin can function as substrate for the enzyme (Kim and Paik, 1970). Even though the biological significance of its reaction or the nature of its substrate in biological systems is not known at present, its ubiquitous and abundant distribution in various mammalian tissues (Kim and Paik, 1971b, Paik *et al.*, 1971, 1972; Liss and Edelstein, 1967) and the existence of a specific natural inhibitor for the enzyme (Kim and Paik, 1971b) suggest a potentially important role for the enzyme in cellular function.

Kinetic studies carried out recently with protein methylase II from erythrocytes showed one of the products, *S*-adenosyl-L-homocysteine, to be a potent inhibitor (Kim, 1974). However, investigations in different laboratories have revealed that the inhibition of methyltransferase systems by *S*-adenosyl-L-homocysteine shows wide variation in potency. While the DNA modification methylase of *E. coli* is not inhibited (Lautenberger and Linn, 1972) several other methylases exhibited greater affinity for *S*-adenosyl-L-homocysteine than for *S*-adenosyl-L-methionine (Deguchi and Barchas, 1971; Coward *et al.*, 1972; Kerr, 1972; Baudry *et al.*, 1973; Lin *et al.*, 1973). Furthermore, Hildesheim *et al.* (1972) showed that some analogs of *S*-adenosyl-L-homocysteine exhibited differential inhibition toward tRNA methylation and protein methylation. As a first step toward understanding the molecular mechanism of these differences quantitative kinetic criteria must be established for methyltransferases. Comparison of Michaelis constants for *S*-adenosyl-L-methionine and inhibition constants for *S*-adenosyl-L-homocysteine become more meaningful when the reaction mechanism is known since the meaning of these constants varies depending on the kinetic mechanism (Cleland, 1963a). With these considerations in mind we designed experiments to determine the kinetic mechanism of calf thymus protein methylase II. Bovine pancreatic ribonuclease was chosen as the protein substrate because of its ready availability and because its complete amino acid sequence is known (Smyth *et al.*, 1963). This communication describes the results of initial velocity and product inhibition studies as well as the results of inhibition studies with adenosine on the enzyme in the forward direction. Because of experimental difficulties in isolating the unstable product, protein methyl ester, the reverse reaction was not studied. This is the first report of kinetic studies on macromolecular methylation using a homogeneous enzyme preparation.

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Experimental Section

Materials. *S*-Adenosyl-L-[methyl-^{14}C]methionine (specific activity, 58 Ci/mol) was obtained from New England Nuclear Corporation. Five times crystallized bovine pancreatic ribonuclease, obtained from Sigma Chemical Co., was used as the protein substrate. *S*-Adenosyl-L-homocysteine was also a product of Sigma. Other chemicals used were obtained from local sources and were of analytical grade.

Protein methylase II purified from calf thymus as described in an earlier paper (Kim, 1973) having a specific activity of 7280 (with denatured calf thymus cytosol protein as substrate) was used. The enzyme was homogeneous as judged by the criteria of Sephadex G-100 column chromatography, analytical ultracentrifugation, and polyacrylamide gel electrophoresis.

Preparation of Ribonuclease Solution. Ribonuclease was dissolved in a minimum volume of chilled 0.05 N NaOH to give a pH of 6.0 and diluted to the desired concentration with chilled water. The molecular weight of RNase was taken to be 13,700.

Enzyme Assay. The assay of protein methylase II was conducted according to the method of Kim and Paik (1970). The incubation mixture contained in a total volume of 0.5 ml: 60 μmol of sodium phosphate and 18.5 μmol of citrate (pH 6.0); 1 μmol of EDTA; 6 μmol of 2-mercaptoethanol; the indicated concentrations of *S*-adenosyl-L-[methyl-^{14}C]methionine and ribonuclease; and enzyme. All components of the reaction mixture except *S*-adenosyl-L-methionine were mixed together in ice and preincubated at 37° for 4 min before initiating the reaction with *S*-adenosyl-L-methionine. The incubation was for 10 min. The reaction was stopped by adding 5 ml of trichloroacetic acid (150 g/l.). The precipitated protein was centrifuged and washed three times with 5-ml portions of trichloroacetic acid and once with 5 ml of ethanol. The precipitate was finally suspended in 0.2 ml of 1.5 N NH_4OH and dispersed in 10 ml of Instagel (Packard) in a scintillation vial and the radioactivity was counted.

Estimation of Protein. The protein concentrations of enzyme solutions were estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Treatment of Data. Initial velocity data were first analyzed graphically by plotting the reciprocals of velocities against the reciprocals of substrate concentrations. When these plots were linear the data were fitted to eq 1 according to the least-squares method of Wilkinson (1961) assuming equal variance for the velocities. Intercepts ($1/V$) and slopes (K/V) of such fits were plotted graphically against the reciprocals of the concentrations of the nonvaried substrate to extract kinetic constants. The nomenclature used in this paper is that of Cleland (1963b). All initial velocities are expressed as cpm per 0.5 ml of reaction mixture; 920 cpm under the experimental conditions correspond to 1 pmol of methyl group incorporated into ribonuclease per min.

$$v = \frac{VA}{KA + A} \qquad (1)$$

Results and Discussion

Initial Velocity Patterns. When ribonuclease was the varied substrate at various fixed concentrations of *S*-adenosyl-L-methionine the initial velocity pattern shown in Figure 1 was obtained. The lines appear to converge at a point on or near the abcissa within the experimental error of the fitted lines. A similarly converging initial velocity pattern was observed also when RNase[1] concentration was kept fixed at various levels and the concentration of *S*-adenosyl-L-methionine was varied. Such converging initial velocity patterns are consistent with a sequential bireactant enzymic mechanism which follows the rate equation of the general form

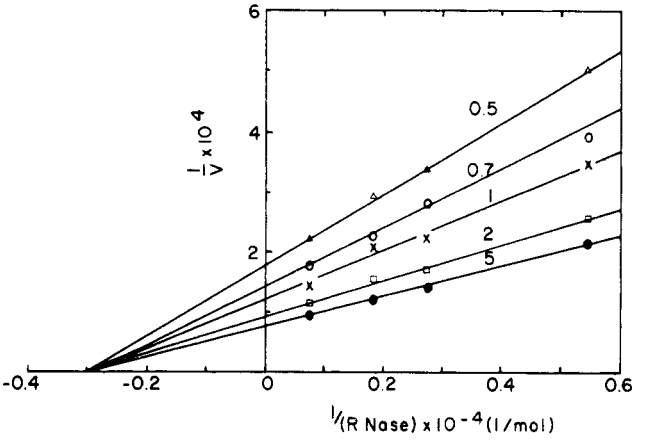


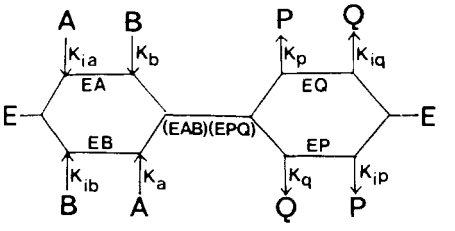
FIGURE 1: Effect of RNase concentration on the calf thymus protein methylase II reaction. RNase concentration was varied from 1.85×10^{-4} to 14.76×10^{-4} M. The numbers above the lines indicate the concentrations of *S*-adenosyl-L-methionine used $\times 10^6$ M. Assay conditions were standard; 1.5 μg of enzyme protein was used.

$$v = \frac{VAB}{K_{ia}K_b + K_aB + K_bA + AB} \qquad (2)$$

where v is the initial velocity, A and B are substrate concentrations, V and K's are constants. In this mechanism both substrates must be present simultaneously on the enzyme surface before any product is released. The same form of rate equation as eq 2 is obtained whether there is an obligatory order of addition of substrates and release of products or not. Consequently converging initial velocity patterns are given by the ordered BiBi mechanism, the Thorell–Chance mechanism, as well as by random mechanisms (Cleland, 1970). However, product-inhibition studies can often be used to make a choice among the various possibilities. In the present studies *S*-adenosyl-L-homocysteine was used as a product inhibitor.

Product Inhibition Patterns. Inhibition by *S*-adenosyl-L-homocysteine was competitive *vs.* ribonuclease (Figure 2) as well as *vs.* *S*-adenosyl-L-methionine (Figure 3). Had addition of substrates to protein methylase II been ordered, inhibition by *S*-adenosyl-L-homocysteine against one of the substrates would have been noncompetitive (Cleland, 1963b). Therefore it would appear that the protein methylase II reaction followed a random sequence of addition of substrates and release of products which may be represented as in Scheme I. A, B, P, and Q in Scheme I stand for *S*-

SCHEME I



[1] Abbreviations used are: RNase, ribonuclease; EDTA, ethylenediaminetetraacetate.

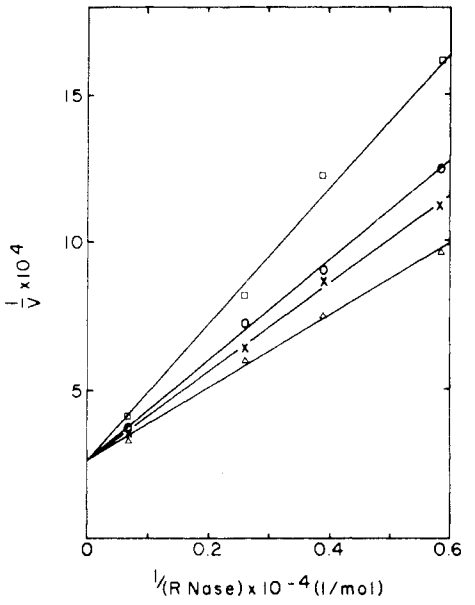


FIGURE 2: Effect of the product, *S*-adenosyl-L-homocysteine, on the calf thymus protein methylase II reaction with RNase as the variable substrate. Concentrations of *S*-adenosyl-L-homocysteine used were: 0, (△), 5 (X), 10 (○), and 20 (□) × 10^{-6} M. The concentration of *S*-adenosyl-L-methionine was 20 × 10^{-6} M. Other conditions were standard; 1 μg of enzyme protein was used.

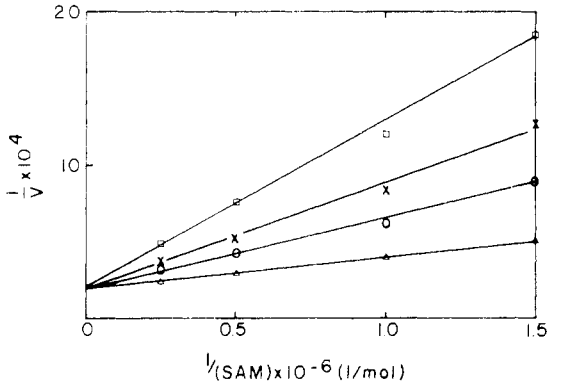


FIGURE 3: Effect of *S*-adenosyl-L-homocysteine on calf thymus protein methylase II reaction with *S*-adenosyl-L-methionine (SAM) as the variable substrate. Concentrations of *S*-adenosyl-L-homocysteine used were: 0 (△), 5 (○), 10 (X), and 20 (□) × 10^{-6} M. RNase concentration was 14.7 × 10^{-4} M; 1 μg of enzyme protein was used. Other conditions were standard.

adenosyl-L-methionine, RNase, RNase methyl ester, and *S*-adenosyl-L-homocysteine, respectively. K_{ia}, K_{ib}, K_{ip}, and K_{iq} are the dissociation constants for the reaction of the free enzyme with A, B, P, and Q, respectively. K_a, K_b, K_p, and K_q represent their respective Michaelis constants, that is, dissociation constants for their reaction with the enzyme saturated with the other substrate or product, respectively. K_a and K_{ib} were calculated from the secondary plots of intercepts and slopes of Figure 1 against the reciprocals of the concentrations of *S*-adenosyl-L-methionine (Figure 4) as described by Cleland (1970). K_b and K_{ia} were calculated similarly from analogous secondary plots of intercepts and slopes, of the initial velocity data with *S*-adenosyl-L-methionine as the variable substrate, against the reciprocals of the concentrations of RNase. The calculated kinetic constants are presented in Table I. In a random bisubstrate enzymic reaction if the rate-limiting step is the interconversion of the central complexes (E-EPQ) and if all other steps

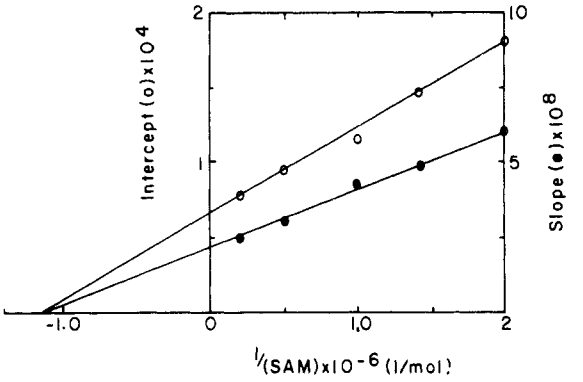


FIGURE 4: Secondary plots of the intercepts and slopes of Figure 1 against the reciprocals of the concentrations of *S*-adenosyl-L-methionine.

Table I: Kinetic Constants of Calf Thymus Protein Methylase II.[a]

Constant	Value (M)
K_a	0.87×10^{-6}
K_{ia}	0.97×10^{-6}
K_b	2.86×10^{-4}
K_{ib}	3.35×10^{-4}

[a] The constants were calculated graphically from the secondary plots of the intercepts and slopes of initial velocity plots as described in the text. K_a and K_b are respectively the dissociation constants for the reaction of *S*-adenosyl-L-methionine and RNase with the enzyme–RNase complex and enzyme–*S*-adenosyl-L-methionine complex. K_{ia} and K_{ib} stand for the dissociation constants for the reaction of *S*-adenosyl-L-methionine and RNase, respectively, with the free enzyme.

equilibrate rapidly, the relationship $K_{ia}K_b = K_{ib}K_a$ holds. The relationship was found to hold in the present studies within experimental error (2.77 × 10^{-10} and 2.91 × 10^{-10} M^2, respectively), suggesting that the rapid equilibrium assumption might be valid for the protein methylase II reaction.

The rate equations for the rapid equilibrium random mechanism, in reciprocal form, in presence of the product Q, with A and B as the variable substrates, are given by eq 3 and 4, respectively.

$$\frac{1}{v} = \frac{K_a}{V}\left\{1 + \frac{K_{ib}}{B}\left(1 + \frac{Q}{K_{iq}}\right)\right\}\frac{1}{A} + \frac{1}{V}\left(1 + \frac{K_b}{B}\right) \quad (3)$$

$$\frac{1}{v} = \frac{K_b}{V}\left\{1 + \frac{K_{ia}}{A}\left(1 + \frac{Q}{K_{iq}}\right)\right\}\frac{1}{B} + \frac{1}{V}\left(1 + \frac{K_a}{A}\right) \quad (4)$$

Equations 3 and 4 predict that the secondary plots of the slopes of the initial velocity plots against the concentrations of *S*-adenosyl-L-homocysteine should be linear. This was found to be true in the present studies. From the horizontal intercepts of these plots apparent inhibition constants (K_{iq} app.) can be calculated (Cleland, 1963a). Equations 3 and 4 predict also that the apparent inhibition constants thus obtained should be functions of the concentration of the nonvaried substrate. This has been found to be so in the

Table II: Values of K_{iq} as Determined from the Competitive Inhibition by *S*-Adenosyl-L-homocysteine with Respect to *S*-Adenosyl-L-methionine (A) (SAM) and RNase (B).[a]

A			B		
Concn of RNase $\times 10^4$ M	K_{iq} app. $\times 10^6$ M	K_{iq} $\times 10^6$ M	Concn of SAM $\times 10^6$ M	K_{iq} app. $\times 10^6$ M	K_{iq} $\times 10^6$ M
3.68	2.2	1.08	5	9.75	1.58
7.35	3.1	1.01			
14.70	5.4	1.00	20	27.25	1.26
	Av	1.03		Av	1.42

[a] Each apparent K_{iq} is the average of three determinations. K_{iq} was calculated by eq 5 or 6.

present studies (Table II). The real K_{iq}'s were calculated from the relations

$$K_{iq}\ \text{app.} = K_{iq}\left(1 + \frac{B}{K_{ib}}\right) \quad (5)$$

$$K_{iq}\ \text{app.} = K_{iq}\left(1 + \frac{A}{K_{ia}}\right) \quad (6)$$

and are presented in Table II.

The observation of competitive inhibition by *S*-adenosyl-L-homocysteine against RNase would suggest that the dead-end complex, enzyme–RNase–*S*-adenosyl-L-homocysteine, is not formed under the conditions employed.

Inhibition by Adenosine. It was expected that compounds possessing those groups of *S*-adenosyl-L-methionine and *S*-adenosyl-L-homocysteine molecules, which might be important in binding to the enzyme, might function as inhibitors. Among L-methionine, L-homocysteine, and adenosine, adenosine alone was found to inhibit significantly when tested at 3 mM concentration. The inhibition by adenosine was linear competitive against *S*-adenosyl-L-methionine as well as against RNase. These results are again in conformity with a rapid equilibrium random mechanism for protein methylase II reaction. The observation of competitive inhibition by adenosine against RNase suggests that the ternary complex, enzyme–RNase–adenosine, is not formed. This is not surprising in view of the fact that *S*-adenosyl-L-homocysteine also was not able to form a ternary complex (Figure 2) under the experimental conditions. It appears that the positive charge on the *S*-adenosyl-L-methionine molecule is important in the formation of the ternary complex.

The inhibition constants of adenosine were calculated from the replot of slopes of the competitive inhibition data against adenosine concentrations. The calculated values are 1.2 mM against *S*-adenosyl-L-methionine and 4.6 mM against RNase.

The results of the present paper are consistent with a random mechanism for the kinetics of protein methylase II reaction. The realization of the relationship $K_{ia}K_b = K_{ib}K_a$ and the observation of competitive inhibition by the product *S*-adenosyl-L-homocysteine, against either *S*-adenosyl-L-methionine or RNase would suggest that the rate-limiting step may be the interconversion of the central ternary complexes and all other steps may equilibrate rapidly. However, experiments on isotope exchange at equilibrium are needed to decide whether the mechanism is truely rapid equilibrium. This is not feasible at present because of the lack of the protein product.

Other methyltransferases, the kinetics of which have been examined also, appear to follow the random mechanism. Phenylethanolamine *N*-methyltransferase of bovine medulla (Connet and Kirshner, 1970) and catechol *O*-methyltransferase of human liver (Ball *et al.*, 1972) show complex kinetic patterns but suggest a random mechanism. The kinetics of catechol *O*-methyltransferase of rat liver (Flohe and Schwabe, 1970), histamine methyltransferase of guinea pig brain (Baudry *et al.*, 1973), and indolethylamine *N*-methyltransferase of rabbit lung (Lin *et al.*, 1973) appear to have kinetic properties similar to those of protein methylase II reported here.

The inhibition constants obtained in the present studies, for *S*-adenosyl-L-methionine and *S*-adenosyl-L-homocysteine which represent the dissociation constants for the reaction of the free enzyme with these reactants, were not much different from each other (0.97×10^{-6} and 1.03×10^{-6} M, respectively). Thus protein methylase II has about equal affinity for *S*-adenosyl-L-methionine and *S*-adenosyl-L-homocysteine. In this respect calf thymus protein methylase II differs from several other methylases reported in the literature (Deguchi and Barchas, 1971; Coward *et al.*, 1972; Kerr, 1972; Baudry *et al.*, 1973; Lin *et al.*, 1973) to show greater affinity for *S*-adenosyl-L-homocysteine than for *S*-adenosyl-L-methionine.

References

Ball, P., Knuppen, R., Haupt, M., and Breuer, H. (1972), *Eur. J. Biochem. 26,* 560–569.

Baudry, M., Chast, F., and Schwartz, J.-C. (1973), *J. Neurochem. 20,* 13–21.

Cleland, W. W. (1963a), *Biochim. Biophys. Acta 67,* 173–187.

Cleland, W. W. (1963b), *Biochim. Biophys. Acta 67,* 104–172.

Cleland, W. W. (1970), *Enzymes, 3rd Ed. 2,* 1.

Connet, R. J., and Kirshner, N. (1970), *J. Biol. Chem. 245,* 329–334.

Coward, J. K. D'Urso-Scott, M., and Sweet, W. D. (1972), *Biochem. Pharmacol. 21,* 1200–1203.

Deguchi, T., and Barchas, J. (1971), *J. Biol. Chem. 246,* 3175–3181.

Flohe, L., and Schwabe, K.-P. (1970), *Biochim. Biophys. Acta 220,* 469–476.

Greenberg, D. M. (1963), *Advan. Enzymol. 25,* 395–431.

Hildesheim, J., Hildesheim, R., Lederer, E., and Yon, J. (1972), *Biochimie 54,* 989–995.

Kerr, S. J. (1972), *J. Biol. Chem. 247,* 4248–4252.

Kerr, S. J., and Borek, E. (1973), *Enzymes, 3rd Ed. 9,* 167.

Kim, S. (1973), *Arch. Biochem. Biophys. 157,* 476–484.

Kim, S. (1974), *Arch. Biochem. Biophys. 161,* 652–657.

Kim, S., and Paik, W. K. (1970), *J. Biol. Chem. 245,* 1806–1813.

Kim, S., and Paik, W. K. (1971a), *Biochemistry 10,* 3141–3145.

Kim, S., and Paik, W. K. (1971b), *Biochim. Biophys. Acta 252,* 526–532.

Lautenberger, J. A., and Linn, S. (1972), *J. Biol. Chem. 247,* 6176–6182.

Lin, R.-L., Narasimhachari, N., and Himwich, H. E. (1973), *Biochem. Biophys. Res. Commun. 54,* 751–759.

Liss, M., and Edelstein, L. M. (1967), *Biochem. Biophys. Res. Commun. 26,* 497–504.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193,* 265–275.
Paik, W. K., and Kim, S. (1971), *Science 174,* 114–119.
Paik, W. K., Lee, W. H., and Lawson, D. (1971), *Exp. Gerontol. 6,* 171–177.
Paik, W. K., Lee, W. H., and Morris, H. P. (1972), *Cancer Res. 32,* 37–40.
Smyth, D. G., Stein, W. H., and Moore, S. (1963), *J. Biol. Chem. 238,* 227–234.
Wilkinson, G. N. (1961), *Biochem. J. 80,* 324–332.

Nicotinamide 3,N^4-Ethenocytosine Dinucleotide, an Analog of Nicotinamide Adenine Dinucleotide. Synthesis and Enzyme Studies†

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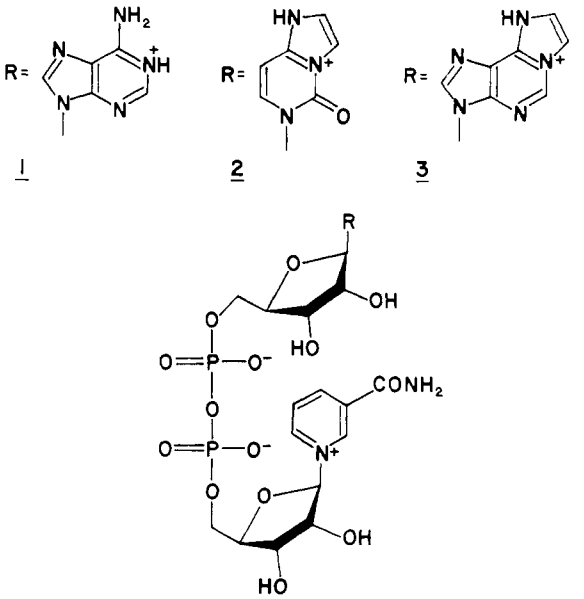
ABSTRACT: A structural analog of NAD^+, nicotinamide 3,N^4-ethenocytosine dinucleotide (ϵNCD^+), has been synthesized, characterized, and compared in activity with the natural coenzyme in several enzyme systems. The V_{max} and apparent K_m values were determined for NAD^+, ϵNCD^+, and ϵNAD^+ (nicotinamide 1,N^6-ethenoadenine dinucleotide) with yeast alcohol, horse liver alcohol, pig heart malate, beef liver glutamate, and rabbit muscle lactate and glyceraldehyde-3-phosphate dehydrogenases. The V_{max} for ϵNCD^+ was as great or greater than that obtained for NAD^+ with three of the enzymes, 60–80% with two others, and 14% with one. ϵNCD^+ was found to be more active than ϵNAD^+ with all six dehydrogenases. ϵNCD^+ served as a substrate for *Neurospora crassa* NADase, but could not be phosphorylated with pigeon liver NAD^+ kinase. NAD^+ pyrophosphorylase from pig liver was unable to catalyze the formation of ϵNCD^+ from the triphosphate derivative of ϵ-cytidine and nicotinamide mononucleotide, but was able to slowly catalyze the pyrophosphorolytic cleavage of ϵNCD^+. The coenzyme activity of ϵNCD^+ with dehydrogenases can be discussed in terms of the close spatial homology of ϵNCD^+ and NAD^+, which may allow similar accommodations within the enzyme binding regions.

The prominent biological roles played by nicotinamide adenine dinucleotide (NAD^+) (**1**) and its reduced form NADH have generated considerable interest in these compounds and in the enzymes which utilize them as coenzymes or substrates. One investigative technique that has been employed in determining structure and function relationships of these compounds has been the preparation of NAD^+ analogs and the study of their interaction with dehydrogenases and other enzymes (Sund, 1968a; Colowick, *et al.,* 1966; Biellmann *et al.,* 1974; Chaykin, 1967; Suhadolnik *et al.,* 1974). Analogs have also been used in studies of the mechanisms of dehydrogenase action (Sund, 1968b), the detection of heterogeneity of enzymes having the same function (Kaplan, 1963a), and the evolution of enzyme structure (Kaplan, 1963b; Kaplan *et al.,* 1960; *cf.* Rao and Rossmann, 1973).



The close spatial outline and similar potential binding areas of the base moiety of ϵ-cytidine nucleotides and adenosine nucleotides (Barrio *et al.,* 1973), as shown in the overlay in Figure 1, has inspired the preparation of nicotinamide ϵ-cytosine dinucleotide (ϵNCD^+) (**2**) which should closely mimic the structural features of the natural coenzyme,

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[1] Abbreviations following the IUPAC–IUB Commission on Biochemical Nomenclature recommendations (*J. Mol. Biol. 55,* 299 (1971)) are used throughout. The abbreviation "ϵ" stands for etheno, so that ϵCyd is 3,N^4-ethenocytidine or 5,6-dihydro-5-oxo-6-β-D-ribofuranosylimidazo[2,1-*c*]pyrimidine (Secrist *et al.,* 1972; Barrio *et al.,* 1972a); ϵCMP, 3,N^4-ethenocytidine 5′-monophosphate; ϵNCD^+, nicotinamide 3,N^4-ethenocytosine dinucleotide and ϵNCDH is the reduced form; ϵNAD^+, nicotinamide 1,N^6-ethenoadenine dinucleotide and ϵNADH is the reduced form (Barrio *et al.,* 1972b); NMN, nicotinamide mononucleotide, PP_i, pyrophosphate.