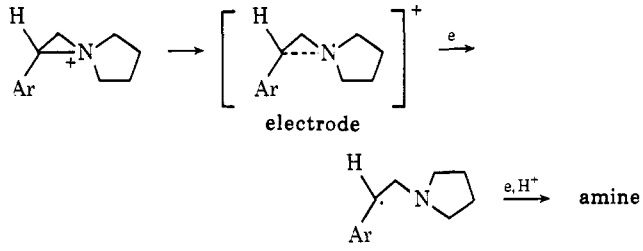
electron transfer, as represented in Scheme I. On the basis of reported work on the *N,N*-dialkylaziridinium salts[2f] and benzyl bromides[16] it seems reasonable to assume two one-electron transfers.[18] This mechanism is consistent with the observed two-electron wave, since in water the radicals produced are more easily reduced than the reactant.

Scheme I



An alternate process involving complete formation of an aminocarbonium ion in a prior equilibrium step is considered less likely at the present time, since $E_{1/2}$ values correlate better with σ values than with σ^+. However, this point is being investigated by measuring $E_{1/2}$ values in aprotic solvents in which the combined wave may be separable.

The present electrochemical results can be compared to other ring opening reactions of aziridinium ions. It was recently reported that 1-ethyl-1-azoniabicyclo-[3.1.0]hexane perchlorate (which cannot form tertiary or benzylic carbonium ions) reacts with nucleophiles exclusively by an SN2 mechanism.[19] However, 2-aryl systems react with water[20] or benzaldehyde[4] *via* an aminocarbonium ion, the same type of species that is reduced by nucleophilic electrons at an electrode surface.

Acknowledgment. The authors express their appreciation to Dr. V. Horak for most useful discussions and to Dr. R. de Levie who helped us perform AC measurements on his instrument. The generous support by the Research Corporation is gratefully acknowledged.

(18) We thank one of the referees for suggesting this process.
(19) C. F. Hammer, S. R. Heller, and J. H. Craig, *Tetrahedron*, **28**, 239 (1972).
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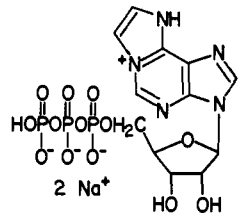
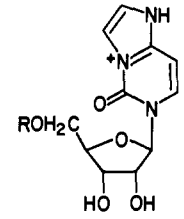
Enzymatic Activity and γ-^{32}P Labeling of Fluorescent Derivatives of Cytidine Triphosphate and Adenosine Triphosphate

Sir:

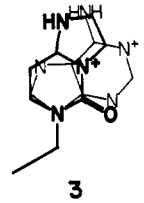
We wish to report a fluorescent derivative of CTP that replaces ATP in certain enzymatic reactions. Chloroacetaldehyde reacts with cytidine to produce a 3,N^4-etheno-bridged derivative.[1] Similarly, we have prepared the corresponding derivatives of the cytidine ribonucleotides, namely, 3,N^4-ethenocytidine 5′-phosphate, or 5′-εCMP (**2a**, shown as the sodium salt), 3,N^4-ethenocytidine 5′-diphosphate (εCDP, **2b**), and 3,N^4-ethenocytidine 5′-triphosphate (εCTP, **2c**).[2] The 1,N^6-etheno derivatives of adenine nucleotides, *e.g.*, εATP (**1**, shown as the disodium salt), are known to replace the corresponding unmodified nucleotides in a number of enzymatic reactions.[3]



1



2a R = $-PO^-Na^+$ (O$^-$)



3

b R = $-POPOH\ Na^+$ (O$^-$ O$^-$)

c R = $-POPOPOH\ 2Na^+$ (O$^-$ O$^-$ O$^-$)

In the enzymatic phosphorylation of 3-phosphoglyceric acid, εCTP[2] is 1.4×10^3 times as active as CTP, is essentially equivalent to ATP, and is a significantly better coenzyme than εATP (**1**). This ability to replace ATP permits the enzymatic synthesis of [γ-^{32}P]εCTP and [γ-^{32}P]εATP by phosphate exchange.

Approximately 1.0–1.6 *M* aqueous chloroacetaldehyde[3d] was used in about 20-fold excess to convert the cytidine nucleotides to the derivatives with a 3,N^4-etheno bridge, by stirring at pH 3.5 at 22–37°, until no starting material was detected on cellulose thin layer chromatograms (Eastman Chromagram cellulose sheets using isobutyric acid–NH_4OH–H_2O, 75:1:24, v/v).[4] Decolorization with charcoal, evaporation to dryness followed by precipitation from aqueous solution with ethanol, and washing the precipitate with ethanol gave pure products.[5] The ε-cytidine nucleotides have fluorescent properties similar to those reported for the corresponding nucleoside[1b] and are indistinguishable in this respect. All show a fluorescence emission maximum at 347 nm under acidic conditions upon excitation at 280 nm. The ability of εATP and εCTP to phosphorylate 3-phosphoglyceric acid catalyzed by yeast 3-phosphoglycerate kinase was compared to that of ATP. The phosphorylation was assayed according to

(1) (a) N. K. Kochetkov, V. N. Shibaev, and A. A. Kost, *Tetrahedron Lett.*, 1993 (1971); (b) J. R. Barrio, J. A. Secrist III, and N. J. Leonard, *Biochem. Biophys. Res. Commun.*, **46**, 597 (1972); (c) N. K. Kochetkov, V. N. Shibaev, and A. A. Kost, *Dokl. Akad. Nauk SSSR*, **205**, 100 (1972).

(2) In the shortened forms of the names, the abbreviation "ε" now generally in use stands for the etheno bridge and is also suggestive of the molar absorbance term and fluorescence emission.
(3) (a) J. A. Secrist III, J. R. Barrio, and N. J. Leonard, *Science*, **175**, 646 (1972); (b) J. A. Secrist III, J. R. Barrio, N. J. Leonard, C. Villar-Palasi, and A. G. Gilman, *Science*, **176**, 279 (1972); (c) J. R. Barrio, J. A. Secrist III, and N. J. Leonard, *Proc. Nat. Acad. Sci. U. S.* **69**, 2039 (1972); (d) J. A. Secrist III, J. R. Barrio, N. J. Leonard, and G. Weber, *Biochemistry*, **11**, 3499 (1972); (e) R. F. Steiner, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, **23**, 139 (1972).
(4) R_f values are: εCMP, 0.54; εCDP, 0.37; εCTP, 0.24 *vs.* CMP, 0.39; CDP, 0.18; CTP, 0.09.
(5) Satisfactory elemental analyses were obtained for all the new compounds mentioned (**2a–c**). If needed, chromatography on DEAE Sephadex using a gradient of ammonium formate (0.5–1.2 *M*) (pH ~3.7) produced excellent purification of the chloroacetaldehyde-modified cytosine derivatives.

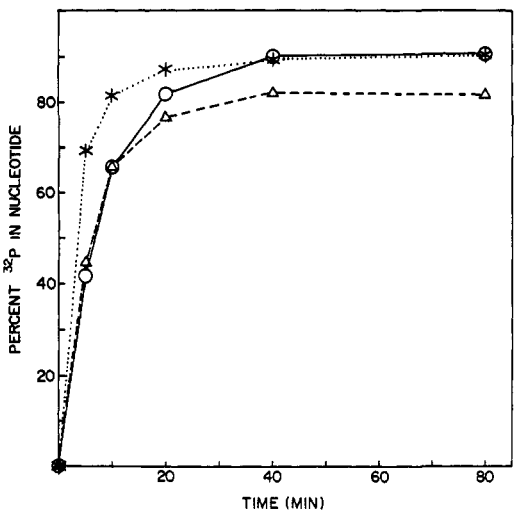


Figure 1. ^{32}P incorporation in the γ phosphate, of ATP (○—○), εATP (**1**) (*···*), and εCTP (**2c**) (△- - -△), in the presence of 3-phosphoglyceric acid, glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase, and $K_2H^{32}PO_4$.

the standard procedure of coupling the reaction to glyceraldehyde-3-phosphate dehydrogenase (muscle).[6] The analogs εATP and εCTP replaced ATP in this system with K_m equal to 3.7 and 0.85, respectively, while under identical conditions the K_m observed for ATP was 0.57. The V_{max} values for εATP and εCTP were equal to 46 and 37% of that of ATP. Under the same conditions[7] CTP did not show any activity, which is in agreement with the finding of Adam.[8,9]

If $^{32}PO_4^{3-}$ is added and NADH omitted from the 3-phosphoglycerate system, the net result is equilibration of the γ (terminal) phosphate in the nucleoside triphosphate with the inorganic phosphate. Using conditions similar to known procedures for preparing [γ-^{32}P]ATP,[10] [γ-^{32}P]εATP and [γ-^{32}P]εCTP were prepared. The nucleoside triphosphate (5 m*M*) was mixed with $K_2H^{32}PO_4$ (0.2 m*M*) in a reaction buffered at pH 8.1 with Tris-HCl (50 m*M*) and containing $MgCl_2$ (6.25 m*M*), dithiothreitol (1.25 m*M*), 3-phosphoglyceric acid (1 m*M*), glyceraldehyde-3-phosphate dehydrogenase (100 μg/ml), and 3-phosphoglycerate kinase (10 μg/ml). The incorporation of ^{32}P into triphosphate was assayed by chromatography of the reaction mixture on polyethylenimine thin layers (Polygram, Brinkmann Instruments) which were developed with 1 *M* LiCl.[11,12] Radioactivities on the chromatograms were measured on a strip scanner (Packard No. 7201). The per cent of the total phosphate incorporated into the triphosphate *vs.* reaction time is plotted in Figure 1. The εATP reached equilibrium most rapidly, even though it phosphorylates 3-phosphoglyceric acid most slowly. Apparently the rate of approach to equilibrium in this complex system is determined by factors other than the phosphorylation rate of 3-phosphoglyceric acid. Traces of the corresponding nucleotide diphosphates formed in the enzymatic reaction showed no incorporation of ^{32}P.

The introduction of the second ring on the cytidine portion of CTP gives the new molecule a spatial outline and binding areas roughly similar to those of the corresponding adenine nucleotide. This relationship is shown by the overlay in formula **3**, in which anti conformations are assumed with respect to the ribosyl phosphate unit, indicated schematically. The same conclusion is supported by preliminary results obtained with εCDP *vs.* ADP as substrates for pyruvate kinase. Under the conditions employed,[3d] εCDP showed activity comparable to ADP, while CDP was considerably less active.[13]

In terms of utility, the availability of γ-^{32}P triphosphates greatly facilitates the enzymatic study of these coenzyme analogs, especially in reactions where phosphate or pyrophosphate is donated to an acceptor. Work along these lines is in progress.

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New Synthetic Methods. Transfer of Chirality from Sulfur to Carbon

Sir:

The synthesis of enantiomerically pure compounds normally involves the resolution of a racemic mixture at some point in a chemical synthesis with the obvious loss of 50% of the material. The question arises as to whether racemic compounds can be converted 100% into enantiomerically pure substances. We wish to report an approach to this problem.

Sulfonium salts possess the interesting property that although they can be obtained optically active,[1,2] the barrier to inversion is sufficiently low to allow racemization in solution at room temperature or slightly above.[2] Thus, resolution of the salt utilizing an optically active anion such as *l*-malate or dibenzoyl hydrogen tartrate crystallizes one diastereomic salt while retaining the other diastereomer in solution. Racemization of this

(6) T. Bücher, *Methods Enzymol.*, **1**, 415 (1955).

(7) The assay mixtures (1 ml) contained 2 m*M* 3-phosphoglyceric acid; 50 m*M* tetramethylammonium *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonate buffer, pH 7.5; 20 m*M* $MgCl_2$; 0.2 m*M* NADH; 100 μg/ml of rabbit muscle glyceraldehyde-3-phosphate dehydrogenase, EC 1.2.1.12 (Boehringer-Mannheim), and 0.1 μg/ml of yeast 3-phosphoglycerate kinase, EC 2.7.2.3 (Boehringer-Mannheim); 0.1–1.0 m*M* for ATP and εCTP; 0.5–5.0 m*M* for εATP.

(8) H. Adam, *Biochem. Z.*, **335**, 25 (1961).

(9) For CTP to show activity equivalent to that of εCTP at 0.15 m*M*, about 70 times higher concentration of substrate and 20 times more enzyme were required.

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(12) R_f values are: phosphate, 0.49; ATP, 0.08; εATP, 0.04; εCTP, 0.17.

(1) K. K. Andersen, *Chem. Commun.*, 1051 (1971).

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