OXY-COENZYME A: A COMPETITIVE INHIBITOR OF COENZYME A IN THE PHOSPHOTRANSACETYLASE REACTION

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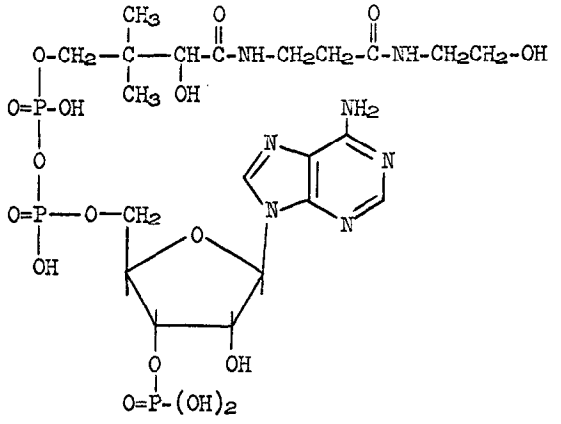
The successful synthesis of Coenzyme A (CoA) by Moffatt and Khorana (1961) and by Michelson (1964) has provided a route for the synthesis of CoA analogs. The oxygen analog, "oxy-coenzyme A" (oxy-CoA), where the -SH group is replaced by an -OH, would be of immediate interest since the oxygen analog of pantetheine, "oxypantetheine" (Stewart, Cheldelin and King, 1955) has been shown to be a competitive growth inhibitor of Lactobacillus helviticus 80.



Oxy-coenzyme A

This report describes the preparation of the 2', 3'-phosphate isomeric mixture of Oxy-CoA from oxypantetheine-4' phosphate and adenosine-2', 3'-cyclic phosphate 5'-phosphoromorpholidate. The results of inhibition studies with the phosphotransacetylase reaction are also presented.

EXPERIMENTAL

Materials and Methods.- Venom phosphodiesterase was purchased from Worthington Biochemical Corporation. Phosphotransacetylase, Lot No. 06155109, was purchased from Boehringer Mannheim Corporation. DEAE-cellulose, Selectacel Standard Type, and ECTEOLA-cellulose, Selectacel Type 40, were purchased from Carl Schleicher and Schuell Company.

Paper chromatography was carried out by the descending technique on Whatman No. 1 paper. The solvent systems used were: Solvent A, n-butyl alcohol-acetic acid-water (5:2:3 v/v); Solvent B, n-propyl alcohol-concentrated ammonia-water (55:10:35 v/v); Solvent C, ethyl alcohol-1 M ammonium acetate, pH 7.5, (7:3 v/v). Adenine containing compounds were located by their characteristic ability to absorb ultraviolet light. Phosphate containing compounds were located by the Phosphate spray reagent (Bandurski and Axelrod, 1951). The R_f's of various compounds are given in Table I.

TABLE I

R_f Values

Compound	Solvent A	Solvent B	Solvent C
Adenosine-5' phosphate	0.24	0.35	0.15
Oxypantetheine-4' phosphate	0.45	0.53	0.53
Adenosine-2'(3'),5' diphosphate	0.15	0.25	0.04
Oxy-CoA	0.16	0.37	0.16
Venom digest of Oxy-CoA	0.15, 0.45	0.25, 0.52	0.04, 0.53

Degradations by venom phosphodiesterase were accomplished by incubating 1.4 mg Oxy-CoA mixed isomers, 100 μmoles tris-HCl buffer

(pH 9.0), and 0.2 mg of enzyme in a total volume of 0.4 ml for 90 min at 38° C. Aliquots were spotted directly on sheets of chromatography paper and developed with the various solvent systems.

Phosphotransacetylase assays were performed by the method of Bergmeyer et al. (1963). The components of the system were: 235 μmoles Tris - HCl buffer, pH 7.4; 1.5 mg glutathione; 8 mg acetylphosphate (lithium salt); 30 μmoles ammonium sulfate; 0.33 μg phosphotransacetylase; 0.134 to 0.670 μmoles CoA: 3.0 ml total volume.

Bis-(4-morpholine N,N'-dicyclohexylcarboxamidinium)-adenosine-2', 3'-cyclic phosphate 5'-phosphoromorpholidate was prepared by the method of Moffatt and Khorana (1961).

Oxy-coenzyme A.- The procedure of Moffatt and Khorana was modified as follows. D-Oxypantetheine-4' phosphate, dilithium salt,* 223 mg (0.48 mmoles), was converted to the pyridinium salt by passage through a 1 X 5 cm Dowex 50W X 8 (pyridinium form) column and evaporating the effluent to dryness in vacuo. Final traces of water were removed by several repeated additions and evaporations in vacuo of anhydrous pyridine. The oxypantetheine-4' phosphate in 10 ml anhydrous pyridine was added to a 3 ml anhydrous pyridine solution containing 222 mg (0.2 mmoles) of bis-(4-morpholine N,N'-dicyclohexylcarboxamidinium)-adenosine-2', 3'-cyclic phosphate 5'-phosphoromorpholidate and the mixture evaporated to a viscous oil in vacuo. After an additional evaporation with anhydrous pyridine, the mixture was dissolved in 10 ml of anhydrous pyridine and permitted to react overnight at room temperature in a tightly stoppered flask. Pyridine was subsequently removed by several evaporations in vacuo with water; the unreacted morpholidate decomposed and the 2', 3'-cyclic phosphate opened by treatment with 10 ml of 0.1 N HCl for 1 hr at room tempera-

* Manuscript in preparation

ture. Several evaporations in vacuo with methyl alcohol were used to remove the HCl. The residue was dissolved in 25 ml of water, the pH adjusted to 6.0 with dilute NH_4OH, and the solution applied to a 2.2 X 30 cm DEAE-cellulose (chloride form) column. The column was washed with water until the effluent was essentially free of UV absorbing material. Then the adsorbed compounds were eluted by application of an acidic lithium chloride linear gradient. The reservoir contained 2.0 liters of 0.15 M LiCl in 0.003 N HCl and the mixing vessel contained 2.0 liters of 0.003 N HCl. Fifteen ml fractions were collected. The oxy-CoA was found in tubes 106-129. This peak was pooled and found to contain 2132 OD units at 259 mμ (72% of the total applied OD units). After adjusting the pH to 4.5 with 1 N LiOH, the peak was evaporated to dryness in vacuo. Lithium chloride was removed from the solid white residue by repeated extraction with 40 ml fractions of methyl alcohol-acetone (1:15). After drying over P_2O_5 in vacuo at room temperature overnight a yield of 89 mg (59%) of the mixed isomers of oxy-CoA was obtained.

In an attempt to separate the 2', 3'-phosphate isomers of oxy-CoA, an analytically pure sample of the mixed isomers was prepared. Oxy-CoA, 1940 OD units, was loaded on a 2.2 X 30 cm ECTEOLA-cellulose (chloride form) column and eluted by an acidic lithium chloride linear gradient. The reservoir contained 2 liters of 0.10 M LiCl in 0.003 N HCl and the mixing vessel contained 2 liters of 0.03 M LiCl in 0.003 N HCl. Tubes 47 - 58 contained the major peak, 1540 OD units, (97% of the total recovered OD units). A white powder was obtained by working up this peak in the manner previously described for the DEAE-cellulose column effluent peak. After drying for 3 hrs at 100° C over P_2O_5 in vacuo, the mixed oxy-CoA isomers were obtained as the trilithium salt trihydrate in a yield of 78 mg.

The UV absorption spectra, at pH 7.0, was determined on a Cary

Model 14 Spectrophotometer: $\lambda_{max.}$ = 259 mμ, $\lambda_{min.}$ = 230 mμ; absorbency ratios, 280/260 = 0.15, 250/260 = 0.81; the extinction coefficient, (calculated from the spectrum of the trilithium salt trihydrate, mol. wt. 823), was $\epsilon_{259\ m\mu} = 14.8 \times 10^{-3}$.

Adenosine:P. Calcd. for $C_{21}H_{33}N_7O_{17}P_3Li_3 \cdot 3\ H_2O$: P, 11.3; A:P = 1:3, Found: P, 11.6; A:P = 1: 3.09.

Anal.* Calcd. for $C_{21}H_{33}N_7O_{17}P_3Li_3 \cdot 3\ H_2O$: C, 30.64; H, 4.78; N, 11.91. Found: C, 30.24, 30.51; H, 4.93, 5.14; N, 12.40.

RESULTS AND DISCUSSION

The 2', 3'-phosphate isomers of oxy-CoA were isolated as a single peak from the reaction mixture by DEAE-cellulose chromatography at a LiCl gradient concentration range of 0.06 - 0.07 M. Under the same conditions, Moffatt and Khorana report the isolation of the mixed CoA isomers as a single peak at a gradient concentration of approximately 0.075 M LiCl. However, the oxy-CoA isomers do not separate on an ECTEOLA-cellulose column under the conditions that Moffatt and Khorana used for the separation of their CoA isomers.

The ability of venom phosphodiesterase to hydrolyze the oxy-CoA preparations, cf. Table I, demonstrates that the oxypantetheine-4' phosphate is linked by a pyrophosphate bond to the adenosine moiety at the 5'-position.

Figure 1, shows the effect of isomeric oxy-CoA on the phosphotransacetylase reaction. It is a competitive inhibitor of CoA, with a $K_I = 6.0 \times 10^{-7}$ M. The $V_{max.}$ of the system, under the experimental conditions used in this investigation, is 0.0138 OD/sec. The K_M of CoA with this commercial preparation is 5.6×10^{-4} M, which is identical to the K_M reported by Bergmeyer et al. for their crystalline material.

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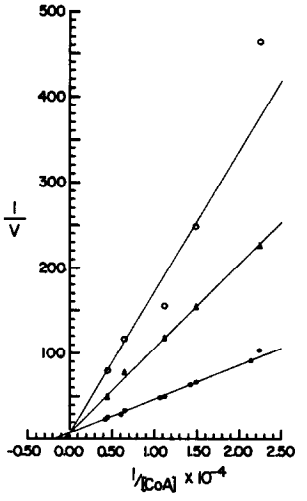

Figure 1. Double reciprocal plot showing competitive inhibition of the phosphotransacetylase reaction by oxy-CoA. The ordinate is secs required to observe an OD change of 0.1, after addition of enzyme.
● --- no added oxy-CoA.
▲ --- 8.1×10^{-7} M oxy-CoA present in reaction.
0 --- 2.03×10^{-6} M oxy-CoA present in reaction.

From these results one can conclude that oxy-CoA is potentially a very useful compound, uniquely suited for the study of CoA dependent reactions. It appears to be the only known antimetabolite specific for CoA.

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