A COENZYME A ANALOGUE, DESULPHO-CoA; PREPARATION AND EFFECTS ON VARIOUS ENZYMES

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The binding of Coenzyme A and its acyl derivatives to carnitine acetyltransferase (EC 2.3.1.7) has been studied in this Department as part of an investigation into the mechanism of action of this enzyme. A CoA analogue lacking the reactive -SH group was needed for this work, and such an analogue would also have general use as a CoA antagonist. The present paper describes the preparation and analysis of desulpho-CoA, in which the thiol group is replaced by hydrogen. The results of a preliminary survey of the effects of this compound on various enzymes are also given.

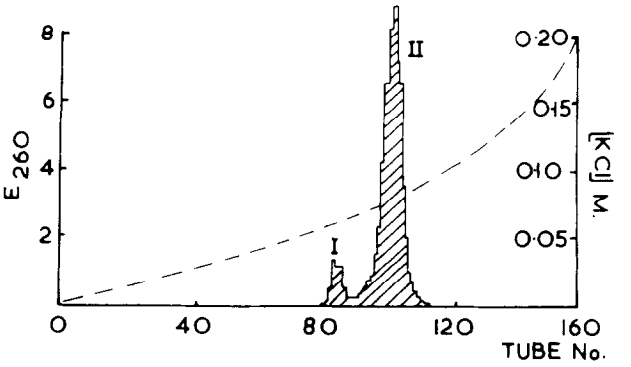
Preparation of Desulpho-Coenzyme A.

Raney nickel is known to remove sulphur from thiol compounds (Mozingo, Wolf, Harris and Folkers, 1943), and its use in converting cysteinyl-s-RNA to alanyl-s-RNA (Chapeville *et al.*, 1962) suggested that it might remove the -SH of CoA without otherwise damaging the molecule.

Raney nickel W-2 was prepared from nickel-aluminium alloy (50% Ni, 50% Al; British Drug Houses Ltd.) by the method of Mozingo (1955), and was stored under absolute ethanol at 4°; before use it was thoroughly washed in glass-distilled water. About 0.5 g. of catalyst (1 ml. of slurry) was added to 56 mg. of CoA.SH (Boehringer und Soehne,

G,m.b.H) dissolved in 4 ml of 0.1 M ammonium acetate (pH 5.1) and 0.5 ml. of saturated disodium ethylenediaminetetra-acetate. The mixture was shaken continuously at 30°, and at intervals 5µl samples were assayed for CoA.SH (Chase and Tubbs, 1966). After 65 min. only 7% of the CoA.SH remained, and the nickel was then removed by centrifuging. After being stored frozen overnight the pale blue solution contained less than 0.5% of the original CoA.SH.

The solution was applied to a column (2 x 35 cm.) of DEAE-cellulose equilibrated with 0.003N-HCl, and eluted with a salt gradient; the mixing vessel initially contained 750 ml. 0.003N-HCl and the reservoir 0.003N-HCl containing 0.2M-KCl. The extinction at 260 mµ of the collected fractions (vol.8.4 ml.) was measured (Fig.1); a blue band,



ELUTION OF DESULPHO-CoA FROM DEAE-CELLULOSE.

Fig 1

presumably containing nickel, was discarded, and the fractions (95-111) constituting Peak II were pooled and freeze-dried.

Analysis of Desulpho-Coenzyme A.

The material of Peak II was shown to be desulpho-CoA on the following grounds:

(1) The absorption spectrum was measured at pH 2.5 and 7.5. In acid there was maximum absorption at 257 mµ, a minimum at 230 mµ, and E_{280}/E_{260} was 0.22. At pH 7.5 the maximum was at 260 mµ, the minimum

at 228 mμ, and E_{280}/E_{260} was 0.16. These figures agree with those for adenine nucleotides (Beaven, Holiday and Johnson, 1955). Assuming that E_{260} for CoA is 16.4 cm.^{2}/μ mole (Stadtman, 1957), the overall yield of desulpho-CoA was 25 μmoles, or about 50%.

(2) Even after incubation with excess reduced glutathione at pH 8.5 no CoA.SH could be detected.

(3) Total phosphate content, as estimated by the method of Allen (1940), was in agreement with that expected for desulpho-CoA (Table 1). No free phosphate was present.

(4) A sample was hydrolysed with 6N-HCl at 110° for 12 hours, and the hydrolysate was applied to a Technicon Automatic Amino-acid Analyser. Other samples of both CoA.SH and desulpho-CoA were oxidized with performic acid (Hirs, 1956) prior to hydrolysis and analysis. The results (Table 1) show that the desulpho-CoA contained adenine, β-alanine, ethylamine and phosphate in the expected proportions 1:1:1:3.

TABLE I

Component estimated		μ moles desulpho-CoA	μ moles component	Component/ adenine
Total phosphate		0.133	0.407	3.06
β-alanine	a)	0.223	0.200	0.90
	b)	0.223	0.226	1.01
Ethylamine	a)	0.223	0.212	0.95
	b)	0.223	0.250	1.12
Taurine	b)	0.223	0	0
		μ moles CoASH		
β-alanine	b)	0.208	0.175	0.84
Ethylamine	b)	0.208	0	0
Taurine	b)	0.208	+	+

a) = acid hydrolysed; b) = performic oxidised and acid hydrolysed

Effects of Desulpho-CoA on Enzyme Reactions.

All the following enzymes were assayed at 30° in a Beckman DK-2 recording spectrophotometer.

Carnitine acetyltransferase. Crystalline pigeon muscle enzyme was assayed at 232 mμ (Chase & Tubbs, 1966) in a system containing 100mM tris-HCl, pH 7.7, and varied acetyl-CoA and l-carnitine. The K_m for acetyl-CoA was 3.4×10^{-5}M; desulpho-CoA was found to compete with this, and to inhibit non-competitively with respect to l-carnitine. The K_i for desulpho-CoA was 2.3×10^{-5}M, a value similar to the K_i (or, when a substrate, K_m) found with CoA.SH.

Phosphotransacetylase (EC 2.3.1.8). The enzyme from Clostridium kluyveri (Boehringer) was assayed at 232 mμ in a system containing: 100mM tris-HCl, pH7.4; 15 mM $(NH_4)_2SO_4$; 2.5mM acetyl phosphate; 1.25mM glutathione; varied CoA.SH and desulpho-CoA. The K_m for CoA.SH was 2×10^{-4}M, and desulpho-CoA competed with this (K_i 3.5×10^{-6}M).

Citrate synthase (EC 4.1.3.7). The pig heart enzyme was assayed at 412 mμ by the method of Srere, Brazil and Gonen (1963) in a system containing: 100mM tris-HCl, pH 7.7; 0.5mM oxaloacetate; 0.125mM 5,5' -dithiobis-(2-nitrobenzoate); varied acetyl-CoA and desulpho-CoA. The K_m for acetyl-CoA was 1.3×10^{-5}M, and desulpho-CoA competed with a K_i of 5.5×10^{-5}M.

β-Hydroxy-β-methylglutaryl-CoA synthase (EC 4.1.3.5) purified (B. Middleton, unpublished) from baker's yeast was assayed at 303 mμ in the presence of: 100mM tris-HCl, pH 8.0; 20mM $MgCl_2$; 1.75×10^{-6}M acetoacetyl-CoA; varied acetyl-CoA and desulpho-CoA or CoA.SH. The K_m for acetyl-CoA was 1×10^{-5}M, and both CoA.SH and desulpho-CoA competed with this (respective K_i values of 3.3×10^{-5} M and 1×10^{-5}M).

α-Ketoglutarate dehydrogenase from pig heart (Sanadi, Littlefield and Bock, 1952) was assayed by following the reduction of acetylpyridine adenine dinucleotide (APAD; Sigma) at 365 mμ in a system containing:

50mM phosphate, pH 7.2; 1mM $MgCl_2$; 0.5mM EDTA: 5 mM α-ketoglutarate; 0.25mg/ml. APAD; varied CoA.SH and desulpho-CoA. The K_m for CoA.SH was too low (less than 10^{-7}M according to Massey, 1960) for accurate measurement; desulpho-CoA competed with K_i about 20-fold higher than K_m.

Neither β-ketothiolase (EC 2.3.1.16) from pig heart nor acyl-CoA synthetase (EC 6.2.1.2) from ox liver showed appreciable inhibition by desulpho-CoA at 1.1 x 10^{-4}M. The respective K_m values for CoA.SH were about 5 x 10^{-5}M and 5 x 10^{-6}M, and the experimental concentrations were in these ranges.

Discussion

In the case of carnitine acetyltransferase there is evidence that the K_m for CoA.SH or acetyl-CoA is equal to the dissociation constant, K_s, of the enzyme-substrate complexes (Chase and Tubbs, 1966; J.F.A. Chase, unpublished); the present results thus indicate that, at least for this enzyme, the binding of desulpho-CoA is quantitatively similar to that of CoA.SH. In the case of phosphotransacetylase K_i for desulpho-CoA is much smaller than the K_m for CoA.SH, while the converse clearly applies in the cases of β-ketothiolase and acyl-CoA synthetase. Binding of desulpho-CoA to α-ketoglutarate dehydrogenase is very strong, but K_i is still considerably higher than the extremely low K_m for CoA.SH. In all probability the discrepancies between K_i for desulpho-CoA and K_m for CoA.SH, except for carnitine acetyltransferase, are mainly due to the K_m in these cases being a kinetic quantity rather than an equilibrium dissociation constant. The possible importance of the -SH group in binding to some enzymes is of course not excluded.

These experiments suggest that desulpho-CoA may be useful in metabolic and mechanistic studies concerning CoA. This is also true of oxy-CoA, in which -SH is replaced by -OH; the synthesis of this compound and its inhibition of phosphotransacetylase have recently been described by Stewart and Miller (1965). Desulpho-CoA, however, has

the advantages that it is much more easily prepared and that it presumably consists only of the "natural" 3'-phosphate, whereas synthetic oxy-CoA is a mixture of the 2' and 3' positional isomers.

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