STEREOCHEMISTRY OF THE OXIDATION AT THE β-CARBON OF BUTYRYL-SCoA

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With the intention of determining the mechanism of the oxidation of butyryl-SCoA by acyl-CoA dehydrogenase, we have determined in a first step the stereochemistry of the hydrogen removal at the β-carbon as well as the isotope effect involved in this oxidation.

S and R butyric-^{3}H-3 acids were prepared from R and S ethanol-^{3}H-1 respectively which in term were prepared by enzymatic reduction either of acetaldehyde-^{3}H-1* by NADH or of acetaldehyde by NAD^{3}H, in presence of alcohol dehydrogenase extracted from yeast [1]. The R and S ethanol-^{3}H-1 were then converted to their brosylates by treatment with *p*-bromobenzenesulphonyl chloride. Alkylation of diethylmalonate anion (formed in dimethyl sulphoxide using sodium hydride as base) by these brosylates gave diethyl ethyl malonate. By saponification, followed by decarboxylation, the S and R butyric-^{3}H-3 acids were obtained. The method of Wieland and Rueff was used for the synthesis of butyryl-SCoA [2]. Butyryl-CoA (R) and (S) had a specific activity of 1.02×10^7 cpm/mmole and 1.43×10^7 cpm/mmole, respectively.

The enzyme was extracted from pork liver [3] and separated from a thiol esterase highly active with the analogous S-butyryl *N*-acetyl cysteamine, but weakly active with the butyryl-SCoA. The oxidation was carried out in 1.0 ml of a solution at pH 8.1 0.1 M in tris ethanolamine acetate and containing 0.1 mg bovine serum albumin, 200 μg phenazine methosulphate, 2,6-dichlorophenolindophenol (2 to 3 molar excess relative to butyryl-SCoA) and about 6.3 μmole of butyryl-SCoA. A solution of enzyme was added to give a total of 0.048 mg of enzyme. The solution was incubated under nitrogen at 30°. The reaction was followed by the rate of reduction of the 2,6-dichlorophenolindophenol ($\epsilon_{600\,nm}$ 21,000) [4]. After lyophilisation, the radioactivity of the water was determined.

The results show that the oxidation of butyryl-SCoA to crotonyl-SCoA is a stereospecific reaction and that the pro-R hydrogen is removed. The possibility of partial racemisation during the chemical synthesis of butyric acid would explain the partial in-

* Prepared by the lead tetraacetate oxidation in water, of sodium lactate-^{3}H-2, followed by lyophilisation.

Table

	% of reaction	Total intitial radioactivity (cpm)	Recovered in water (cpm)	Incorporation %
S Butyryl-^{3}H-3 SCoA	100	5,500	940	17
	67	32,000	1,970	6
R Butyryl-^{3}H-3 SCoA	100	11,800	9,700	82
	63	12,800	6,520	51
	42	8,950	3,140	35

corporation of ^{3}H in the water during the oxidation of S Butyl-^{3}H-3 SCoA.

The observed primary isotope effect is close to 1 (the small differences found are not significant): therefore the rupture of the C-3-H bond of the butyryl-SCoA does not determine the reaction rate. This is similar to the oxidation of stearic acid to oleic acid where the isotope effect for the rupture of the C-9-H bond is also to 1 [5], and the oxidation of succinic acid to fumaric acid by the succinodehydrogenase where the isotope k_H/k_D is 1.35 [6].

References

[1] H.Weber, J.Seibl and D.Arigoni, Helv. Chim. Acta 49 (1966) 741; R.U.Lemieux and J.Howard, Can. J. Chem. 41 (1963) 308.
[2] Th.Wieland and L.Rueff, Angew. Chem. 165 (1953) 1887.
[3] H.Beinert in: Methods in Enzymology, eds. S.P.Kolowick and N.O.Kaplan, Vol. 5, (Academic Press, New York, 1962) p. 546.
[4] H.Beinert and E.Steyn-Parve, J. Biol. Chem. 233 (1958) 843.
[5] G.J.Schroepfer and K.Bloch, J. Biol. Chem. 240 (1963) 54.
[6] J.Retey, J.Seibl, D.Arigoni, J.W.Cornforth, G.Ryback, N.P.Zeylemaker and C.Veeger, Chimia 24 (1970) 33.