Oxidation of Cholesterol by Rat Liver Mitochondria: Influence of Deuterium Oxide

Deuterium oxide (D_2O) has been shown to affect hepatic lipogenesis in the mouse[1] and also to influence the lipid composition of cells grown in tissue culture[2,3]. In an effort to extend the observations of the influence of D_2O on lipid metabolism we have studied the effects of D_2O upon the oxidation of cholesterol and sodium octanoate by rat liver mitochondria. The effect of D_2O upon rat liver enzymes studied in vitro ranges from 65% inhibition to 31% enhancement[4].

Incubations were carried out in the manner described earlier[5,6]. The incubation mixture consisted of 1 ml of a rat liver mitochondrial preparation fortified with 1 ml of a solution containing ATP (25 mg), NAD (5 mg), AMP (8 mg), reduced glutathione (15 mg), Na citrate · H_2O (30 mg), Mg $(NO_3)_2$ · $6H_2O$ (10 mg), potassium penicillin G (2000 units), and streptomycin sulfate (1 mg); 5 ml of a solution of labeled substrate in 0.25 *M* tris (hydroxymethyl)aminomethane · HCl, pH 8.5 and 5 ml of boiled supernatant obtained during the preparation of the mitochondrial suspension. The reaction vessel was a stoppered 125 ml Erlenmeyer flask containing a center well in which there was 2.0 ml of 2.5 *N* NaOH. The flasks were shaken for 18 h at 37°C. The $^{14}CO_2$ evolved during the reaction was trapped in the base from which it was precipitated as $Ba^{14}CO_3$ and assayed for radioactivity by liquid scintillation spectrometry. The contents of the flasks were pooled and the residual steroids extracted with ethyl acetate. One series of incubations was carried out in aqueous medium and in two others, mitochondria from the same pool were added to flasks in which all the solutions had been prepared in D_2O. In view of the report[7] that the pH of a solution containing D_2O is actually higher than the value obtained when an ordinary glass electrode is used according to the formula pD = pH + 0.4, the incubations in which D_2O was used were carried out at two different pH's, 8.1 and 8.5. Parallel experiments were performed using mitochondria prepared from both male and female rat liver. Two different substrates were used, cholesterol-26-^{14}C and sodium octanoate-1-^{14}C. The results of this study are presented in the Table.

It is evident that in D_2O at pH 8.5 the oxidation of both substrates is markedly inhibited. The oxidation of octanoate in D_2O at pH 8.1 is roughly equivalent to that observed at the higher pH, but oxidation of cholesterol in D_2O at pH 8.1 virtually ceases.

In contrast to our earlier reports[8,9] the extent of oxidation of cholesterol-26-^{14}C to $^{14}CO_2$ by male rat liver mitochondrial preparations was not much lower than the oxidation of this substrate by similar preparations from female rat livers. This discrepancy was due to a high level of oxidation by the male preparations in one of the experiments. In another series of three incubations average cholesterol oxidation was 2.7% for preparations from male rat livers and 10.0% for female rat liver preparations, confirming our earlier findings.

The effect of D_2O upon cholesterol oxidation may be due to inhibition of the nuclear changes which are required before side chain oxidation may proceed or may be due to inhibition of scission of the cholesterol side chain. The neutral sterols were extracted from the incubation residues and subjected to thin layer chromatography on silica gel G using two different systems: petroleum ether-ethyl ether-methanol-acetic acid (70:30:8:1) or benzene-ethyl acetate (3:4). In both systems, the major radioactive spot obtained from all the incubation experiments corresponded to a mixture of 25- and 26-hydroxycholesterol, compounds known to be the initial products resulting from mitochondrial oxidation of cholesterol[10,11]. We may then assume that normal nuclear oxidation of cholesterol takes place in D_2O but the rate of conversion may be slower and that, coupled with inhibition of scission of the side chain, results in the observed reduction in the extent of cholesterol oxidation[12].

Oxidation of cholesterol-26-^{14}C and octanoate-1-^{14}C by rat liver mitochondria[a] (average of 5 experiments)

Solvent	pH	Males		Females	
		Cholesterol	Octanoate	Cholesterol	Octanoate
H_2O	8.5	8.0 ± 4.0[b]	37.5 ± 12.3	8.9 ± 3.7	45.8 ± 21.7
D_2O	8.1	0.7 ± 0.3	20.8 ± 14.3	0.6 ± 0.3	22.8 ± 12.7
D_2O	8.5	2.5 ± 1.2	26.5 ± 10.7	3.5 ± 3.7	24.4 ± 8.5

[a] $^{14}CO_2$ as $Ba^{14}CO_3$/substrate-^{14}C · 100. Corrected for mg N. [b] Standard deviation.

Résumé. L'oxydation du cholestérol-26-^{14}C et de l'octanoate de sodium-1-^{14}C par une préparation de mitochondries de foie de rat a été effectuée en milieu aqueux à un pH de 8,5 et dans l'eau lourde (D_2O) à un pH de 8,1 et 8,5. La présence d'eau lourde a inhibé l'oxydation des deux substrats. L'oxydation du cholestérol dans l'eau lourde à un pH de 8,5 a été réduite de 60% à 70% de la valeur normale et de plus de 90% dans l'eau lourde à un pH de 8,1. Aux deux valeurs de pH, l'oxydation de l'octanoate a été réduite de 50% à 70%.

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