The Reversibility of the Δ^8 -Cholesterol- Δ^7 -Cholesterol Isomerase Reaction in Cholesterol Biosynthesis

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The incubation of Δ^7 -cholesterol with a 10000g supernatant or 105000g microsomes in the presence of tritiated water is studied. The reisolated Δ^7 -cholesterol contained up to 0.67 g. atom of tritium/mole. This result can best be explained by assuming the reversibility of the reaction Δ^8 -cholesterol $\rightleftharpoons$ Δ^7 -cholesterol.

One of the enzymic reactions in the biosynthesis of cholesterol is the isomerization of the Δ^8 -double bond to the Δ^7 -position. The enzyme system carrying out this isomerization has been isolated

from the microsomal fraction of rat liver, and the work of Gaylor, Delwiche & Swindell (1966) has established several aspects of the substrate specificity of the enzyme. It was of interest from the

Scheme 1.

mechanistic point of view to gain information on the reversibility of the reaction. Previous attempts using g.l.c. failed to show the formation of Δ^8 -cholestenol (I) from the incubation of Δ^7 -cholestenol (II) with the enzyme system. We have now studied the reversibility of the reaction $(I) \rightleftharpoons (II)$ (Scheme 1) by using a sensitive isotopic technique. It has already been shown (Akhtar & Rahimtula, 1968) that in the biological conversion of Δ^8 -cholestenol (I) into Δ^7 -cholestenol (II) the hydrogen atom at C-9 of compound (II) originates from the protons of the medium.

In the event of the reversibility of the Δ^8 -cholestenol $\rightleftharpoons$ Δ^7 -cholestenol reaction the incubation of Δ^7 -cholestenol (II) in the presence of tritiated water should result in the incorporation of radioactivity at position C-9 of this compound. The present work shows that this is the case.

RESULTS AND DISCUSSION

When Δ^7 -cholestenol (II) was incubated with a 10000g supernatant or 105 000g microsomes of rat liver in the presence of tritiated water under anaerobic conditions the reisolated Δ^7 -cholestenol (II) contained up to 0.67g.atom of tritium/mole (Table 1). Degradation of Δ^7 -cholestenol (II) to

3 β -acetoxy-8,9-epoxycholestan-7-one (VI) and 3 β -acetoxy-8,14-epoxycholestan-7-one (VII) (Table 2) revealed that at least 96% of the radioactivity was incorporated at C-9 of Δ^7 -cholestenol (II).

Thus it may be concluded that there is an enzyme present in the 10000g supernatant and sedimented at 105 000g that catalyses the introduction of a proton at C-9 of Δ^7 -cholestenol (II). This incorporation can be best explained by the enzymic isomerization of the Δ^7 -double bond to the Δ^8 -position followed by the reversion of the double bond to the Δ^7 -position.

Mechanism. The chemical reactions of double bonds occurring through ionic intermediates have been rationalized in terms of a mechanism that normally involves an initial addition of a positive species to the more electron-rich carbon atom of the double bond. Evidence has been provided (Wilton, Munday, Skinner & Akhtar, 1968; Akhtar, Rahimtula, Watkinson, Wilton & Munday, 1969) that a similar electrophilic addition constitutes the crucial event in the transformation of double bonds in biological reactions. The simplest mechanism for the isomerization of the Δ^8 -double bond to the Δ^7 -position would involve the initial electrophilic addition of a proton from the group B of the enzyme to the C-9 of the steroid molecule, thus

Table 1. *Anaerobic incubation of Δ^7 -cholestenol with a 10000g supernatant or 105 000g microsomes from rat liver homogenate in the presence of tritiated water*

Samples (5 ml.; 1g. equivalent of liver/1.5 ml.) of the liver fraction were incubated for 3.5 hr. (Expt. 1) and 5 hr. (Expts. 2, 3 and 4) in the presence of tritiated water (0.5 ml.; approx. 50 mc; radioactivity of the medium in Expts. 2, 3 and 4 was 9.22×10^4 c.p.m./ μ g.atom) under anaerobic conditions.

Expt. no.	Conditions	Total radioactivity incorporated (c.p.m.)	^3H incorporated (g.atom/mole of II)
1a	10000g supernatant + 1 mg. of Δ^7 -cholestenol	84 990	0.3–0.4
1b	As in Expt. 1a but with no Δ^7 -cholestenol	180	—
2a	10000g supernatant + 500 μ g. of Δ^7 -cholestenol	79 785	0.67
2b	As in Expt. 2a but with no Δ^7 -cholestenol	1204	0.01
3a	105 000g microsomes + 500 μ g. of Δ^7 -cholestenol	29 440	0.25
3b	As in Expt. 3a but with no Δ^7 -cholestenol	576	0.005
4a	10000g supernatant + 500 μ g. of Δ^7 -cholestenol	44 186	0.37
4b	As in Expt. 4a but with boiled supernatant	1016	0.009

Table 2. *Degradation of radioactive Δ^7 -cholestenol obtained from Expt. 1a of Table 1*

Compound	Radioactivity (c.p.m./m-mole)
Δ^7 -Cholestenyl acetate (V)	25 900
3 β -Acetoxy-8,9-epoxycholestan-7-one (VI)	1140
3 β -Acetoxy-8,14-epoxycholestan-7-one (VII)	27 200
Radioactivity (%)	
	C-9 C-14
	96 0

giving a carbonium ion (III) at C-8, which then will be neutralized by a proton elimination from C-7. This is shown in the sequence (I) $\rightarrow$ (III) $\rightarrow$ (II).

The removal of the C-7 proton from the carbonium ion of the type (III) may in principle be achieved by either a weak or a strong base. The present work showing the reversibility of the isomerization reaction would require that in the conversion of the Δ^7 -compound into the Δ^8 -compound the initial step once again must be the protonation, this time at C-7, to give the intermediate (III); the conversion of this compound (III) into Δ^8 -cholestenol will then involve the removal of the C-9 hydrogen atom by the group B of the enzyme. The reversibility of the reaction (I) $\rightleftharpoons$ (II) suggests that, to carry out their function effectively, both the enzyme groups A and B should be able to act primarily as acids.

The alternative that tritium is incorporated at C-9 by a process (II) $\rightarrow$ (IV) is possible, but unlikely, since such a reaction involving cleavage of a C-H bond next to an ethylenic linkage is mechanistically unfavourable.

EXPERIMENTAL

Materials. For details see Wilton et al. (1968).

Preparation of homogenates and incubation procedure. The excised livers from male Wistar albino rats (200–300 g.) were homogenized in cold 0.1 M-sodium phosphate buffer, pH 7.1, containing 0.03 M-nicotinamide and 4 mM-MgCl₂ (1.5 ml./g. of liver), and centrifuged at 10000g for 15 min. The 10000g supernatant was either used as such or centrifuged again at 105000g for 1 hr. The microsomal pellet was resuspended in the buffer to a concentration of 1 g. equivalent of liver/1.5 ml.

of buffer. In Expt. 4 of Table 1 a female Lister hooded rat (200 g.) was used. The Δ^7 -cholestenol (II) was solubilized in 50 mg. of Tween 80.

Isolation and purification of Δ^7 -cholestenol. Incubations were terminated by the addition of methanolic 10% KOH (5 ml.), and Δ^7 -cholestenol (40 mg.) in acetone (5 ml.) was added. After saponification at 60° for 5 min. the Δ^7 -cholestenol was extracted with a total of 100 ml. of light petroleum (b.p. 40–60°)–diethyl ether (1:1, v/v) and crystallized twice from methanol. Typical specific radioactivities of the Δ^7 -cholestenol during purification were: first crystallization, 835000 c.p.m./m-mole; second crystallization, 820000 c.p.m./m-mole; third crystallization, 815000 c.p.m./m-mole. Acetylation of Δ^7 -cholestenol gave Δ^7 -cholestenol acetate (V), 825000 c.p.m./m-mole.

Measurement of radioactivity. Samples were dissolved in 0.8% 2-(4-*tert.*-butylphenyl)-5-(4-biphenyl)-1-oxo-3,4-diazole in toluene. All samples were counted in a Beckman 200 CPM or an Intertechnique SL 40 liquid-scintillation counter at about 50% counting efficiency. Quenching was corrected by the use of external standards.

Preparation of 3 β -acetoxy-8,9-epoxycholestan-7-one (VI) and 3 β -acetoxy-8,14-epoxycholestan-7-one (VII). These were prepared by oxidation with CrO₃ of Δ^7 -cholestenyl acetate (V) as described by Akhtar & Rahimtula (1968).

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