Mechanism of Reduction of Double Bonds in Biological Systems: Conversion of Desmosterol into Cholesterol

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Summary The reduction of the 24,25-double bond in cholesterol biosynthesis occurs with the addition of a hydrogen atom from the medium at C-24 and another from the 4-position of NADPH at C-25.

STUDIES carried out on the biological conversion of 7-dehydrocholesterol (II) into cholesterol (I) led us to suggest[1,2] a general mechanism for the enzyme-catalysed reduction of C=C, a Markownikoff-type process involving the initial electrophilic addition of an enzyme-bound proton to the more electron-rich terminus of the olefin, thus giving a carbonium ion; the latter ion then being neutralized by the delivery of an hydride from the 4-position of pyridine nucleotide. The hypothesis has received support from subsequent work carried out on the biological reduction of some conjugated dienes involved in steroid biosynthesis.[3] However, the key requirement of the hypothesis was to determine the orientation of addition of hydrogen atoms from NADPH and from the medium to an isolated unsymmetrical double bond.

We report on the reduction of the 24,25-double bond in the conversion of desmosterol (III) into cholesterol (I). The degradations used in the present work were made possible by a recent work in which it was shown that incubation of cholesterol with *Tetrahymena pyriformis* results in the efficient formation of sterols containing 22,23-double bond.[4]

The incubation[2] of desmosterol (III) (1 mg.) with a 105,000 *g* microsomal fraction of rat liver in the presence of [4-$^3H_2$]-NADPH (7 mg., $1{\cdot}2 \times 10^7$ c.p.m.) gave cholesterol containing 3×10^4 c.p.m. Incorporation of radioactivity from [4-$^3H_2$]-NADPH into cholesterol has been observed previously.[5] The biosynthetic cholesterol was mixed with [26,27-^{14}C]cholesterol and the resulting material

(^3H : ^{14}C ratio 1·00) was incubated with the whole cells of *Tetrahymena pyriformis* and the derived cholesta-5,7,22-trien-3β-ol (IV) was cleaved between 22,23-double bond† with potassium carbonate–sodium periodate–potassium permanganate to give isovaleric acid,[4] which was then converted into methyl isovalerate (^3H : ^{14}C ratio 0·92). The latter ester was refluxed with sodium methoxide in methanol for 18 hr. to allow the exchange of the α-hydrogen atom of the ester with the protons of the medium: the recovered methyl ester had ^3H : ^{14}C ratio 0·91. Under the equilibration condition used above, a synthetic sample containing tritium in the α-position of methyl isovalerate lost 90% of its radioactivity. The complete retention of the tritium radioactivity of the biosynthetic cholesterol in the equilibrated methyl isovalerate suggested that the NADPH-derived hydrogen atom was present in the fragment containing carbon atoms 24,25,26, and 27 of cholesterol; the tritium, however, was not located at C-24. We therefore deduce that NADPH-derived hydrogen atom must be located at C-25 of cholesterol.

In an alternative experiment, desmosterol was biologically converted into cholesterol in the presence of tritiated water (the medium had 1·7 mc of tritium per mg.-atom of hydrogen) and nonradioactive NADPH. The biosynthetic cholesterol contained $4·2 \times 10^5$ c.p.m.; a control experiment which lacked the substrate had incorporated less than 1% of the activity of the experimental flask. The biosynthetic cholesterol (^3H : ^{14}C ratio 1·00) was processed as above to give methyl isovalerate (^3H : ^{14}C ratio 0·82) which had lost about 18% of the tritium radioactivity of the precursor cholesterol. This loss may be attributed to the base-catalysed equilibration of the hydrogen atom of isovaleraldehyde which may participate as an intermediate in the oxidation of (IV) into isovaleric acid. The methyl isovalerate (^3H : ^{14}C ratio 0·82), after being refluxed with sodium methoxide–methanol, lost the entire tritium radioactivity (^3H : ^{14}C ratio less than 0·03). These results conclusively prove that in the biological conversion (III) → (I) in the presence of tritiated water the radioactivity that is incorporated in the fragment containing carbon atoms 24,25,26, and 27 of cholesterol, is exclusively located at C-24.

These experiments, showing that in the conversion of desmosterol into cholesterol a hydrogen atom from the medium is incorporated at C-24 and suggesting that another from the 4-position of NADPH is transferred to C-25, strongly support the previously suggested mechanism for the reduction of C=C in biological systems.[1,2] The present work, coupled with a recent report, further suggests that the proton-source-derived hydrogen atom occupies the 24-*pro*-*S*-orientation in cholesterol.[6] The origin of hydrogen atoms at C-24 and C-25 is shown in the partial structure (V).

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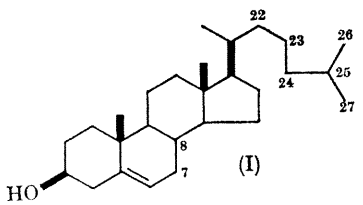
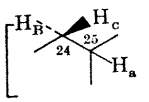
(II) 7,8-Didehydro-(I) H_a from NADPH
(III) 24,25-Didehydro-(I) H_b from proton source
(IV) 7,8; 22,23-Tetradehydro-(I) H_c from mevalonic avid



(I)



(V)

† Cholesterol (40 mg.) was incubated and processed[4] to give after chromatography a mixture of sterols containing up to 10 mg. of cholesta-5,7-22-trien-3β-ol. This mixture, which was contaminated with some starting cholesterol, was subjected to oxidation without further purification.

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[2] D. C. Wilton, K. A. Munday, S. J. M. Skinner, and M. Akhtar, *Biochem. J.*, 1968, **106**, 803.
[3] (a) M. Akhtar, A. D. Rahimtula, I. A. Watkinson, D. C. Wilton, and K. A. Munday, *Chem. Comm.*, 1969, 149; (*b*) Unpublished work in this laboratory has shown that the reduction of the 14,15-double bonds of 4,4-dimethylcholesta-8,14-dien-3β-ol and cholesta-7,14-dien-3β-ol occurs through the addition of H^+ to C-15 and a hydrogen atom from NADPH to C-14.
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