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MAST

BASIC STUDIES OF STEROID HORMONE METABOLISM

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ABSTRACT

Calculations based on several established pathways leading from cholesterol to pregnenolone account for only a small fraction of the total, experimentally found, pregnenolone, when calculated for an obligatory 20- or 22-hydroxy intermediate. Re-evaluation of our kinetic data may provide reasons for keeping the model cholesterol $\rightarrow$ (22 β)-22-hydroxycholesterol $\rightarrow$ (20 β ,22 β)-20,22-dihydroxycholesterol.

BRIEF SUMMARY

Synthesis of Isotopically Labeled Steroids

Studies involving the biosynthesis, secretion rates, catabolic studies and the elaboration of micro-analytical procedures required the syntheses of numerous ^{14}C and ^3H labeled steroids which are commercially not available. The nature and use of these compounds is indicated in the next paragraph.

Biosynthesis of 3 β -Hydroxypregn-5-en-20-one from Cholesterol

Continuing our quantitative studies of the reaction sequences of the transformation involved in the overall reaction cholesterol $\rightarrow$ pregnenolone, the kinetics of metabolism of (20 β)-20-hydroxycholesterol, (22 β)-22-hydroxycholesterol and (20 β ,22 β)-20,22-dihydroxycholesterol with adrenocortical heme protein P-450 preparations was studied at low substrate concentrations. When the substrate was added last to the complete system, first order kinetics were observed. When the reaction was started by the addition of TPNH after the substrate had been equilibrated with the heme protein P-450 system, approximate two phase kinetics appeared to obtain with a much higher initial rate. From the parameters of the disappearance curves obtained after equilibration, the approximate first order rate constants of the interaction of the substrate with the enzyme and of the reaction of the enzyme-substrate complex to yield product could be obtained. The latter rate was considerably higher than the rates of equilibration. The kinetics of the conversion of cholesterol to pregnenolone followed a similar pattern. The access of the substrate to the enzyme appeared to be the rate limiting step in these transformations.

A correlative study was made between the enzymatic rates of conversion of cholesterol to pregnenolone and the substrate-induced difference spectra (in the 390- to 420 nm range) observed with cholesterol-oxygenated derivatives using various heme protein P-450 preparations from bovine adrenocortical mitochondria. With acetone-dried powder extracts at 24 μM (22 β)-20 α ,22-dihydroxycholesterol, 20 α -hydroxy-22-oxocholesterol, 20 β -hydroxy-20-isocholesterol, and 25-hydroxycholesterol exhibited type I difference spectra. Type II spectra were observed with 20 α -hydroxycholesterol (22 β)-22-hydroxycholesterol, (22 β)-22-hydroxycholesterol, 20 α ,21-dihydroxycholesterol, (22 β)-20 α ,22-dihydroxycholesterol, and 22-oxocholesterol. With some acetone-dried powder preparations no difference spectra were observed with (22 β)-20 α ,22-dihydroxycholesterol. The type of the difference

spectra observed with some of the sterols depended on the technique used in the preparation of the heme protein P-450 fractions. There appeared to be no consistent correlation between the ability of the preparations to catalyze the conversions of cholesterol to pregnenolone and the magnitude of the observed difference spectra. Although with the acetone-dried powder preparations the apparent binding affinity of the (20R)-22-hydroxycholesterol and that of the (22R)-20 α ,22-dihydroxycholesterol (as determined from the difference spectra) was higher than that exhibited by 20 α -hydroxycholesterol, the converse was seen with some of the other partially purified heme protein P-450 preparations studied. These results suggested that great caution must be exercised in deriving mechanistic enzymatic conclusions from substrate-induced difference spectra as these may drastically vary both with respect to magnitude and affinity without significantly affecting, in certain cases, the enzymatic activities.

In order to test quantitatively whether cholesterol is an obligatory precursor of pregnenolone we have prepared desmosterol-1,2-³H and, for the first time, the following hypothetical metabolites: (20R)-20-hydroxydesmosterol, (22R)-22-hydroxydesmosterol and (20R,22R)-20,22-dihydroxydesmosterol. In addition we prepared 5-hydroxy-2-methylpent-2-ene and 2-methylpent-2-en-5-one.

A stereospecific synthesis of (20S,22R)- and (20S,22S)-17 α ,20,22-trihydroxycholesterol has been carried out and it could be shown that neither is a metabolite of cholesterol.

The use of radical trapping agents, such as dibenzyl disulfide, in conjunction with an incubation of cholesterol-25,26-³H did not yield any benzyl-isohexyl sulfide-³H. Although the half life of the radical, and its concentration in the steady state and the high reactivity of H $\cdot$ are pertinent, this negative experiment is in agreement with our earlier observations of a 1:1 stoichiometric relationship between the C₂₁ steroid and the sum of the C₆ compounds formed.

List of Publications

1. Substrate-induced Difference Spectra and Cholesterol to Pregnenolone Conversion with Adrenal Heme Protein P-450. S. Burstein, N. Co., M. Gut, H. Schleyer, D. Y. Cooper, and O. Rosenthal, *Biochem.* 11, 573 (1972).
2. Stereospecific Synthesis of (20S,22R)-17 α ,20,22-trihydroxycholesterol and (20S,22S)-17 α ,20,22-trihydroxycholesterol. R. C. Nickolson, and M. Gut, *J. Org. Chem.*, Sept. issue 1972.
3. Kinetic Studies on Substrate-Enzyme Interaction in the Cholesterol Side Chain Cleavage System. S. Burstein, J. Dinh, N. Co., M. Gut, H. Schleyer, D. Y. Cooper, and O. Rosenthal, *Biochem.*, in press.