STEREOCHEMISTRY OF ENZYMIC C-4,5 DEHYDROGENATION OF STEROIDS

Yusuf J. Abul-Hajj

College of Pharmacy, University of Minnesota
Minneapolis, Minnesota

Received March 26, 1971

SUMMARY: Dehydrogenation of 5α-3-ketosteroids with cell-free preparations of Nocardia restrictus involves a cis removal of the 4α and 5α hydrogens while dehydrogenation of 5β-3-ketosteroids proceeds by a stereospecific removal of the 4α and 5β hydrogens.

A variety of microorganisms have been shown to be capable of catalyzing the introduction of double bonds into the 1,2 and 4,5 positions of the A/B trans or A/B cis steroids (1,2). It is now generally accepted that the enzymes involved in these dehydrogenation reactions, namely, Δ^1-dehydrogenase, Δ^4-5-α-dehydrogenase and Δ^4-5β-dehydrogenase, are flavoprotein enzymes (3,4,5) that act by a direct dehydrogenation, rather than that of a hydroxylation followed by a dehydration, mechanism as observed for succinic dehydrogenase (6) fatty acyl dehydrogenase (7) and dihydroorotic dehydrogenase (8).

The purpose of the present investigation was to determine the stereochemical course of bacterial C-4,5 dehydrogenations of steroids. With this goal in mind, we prepared various steroid substrates substituted at the 4α and 5β positions. The compounds prepared were 4α-methyl-5α-androstane-3,17-dione, 4β-methyl-5β-androstane-3,17-dione, 4α-bromo-5α-androstane-3,17-dione, 4β-methyl-5β-androstane-3,17-dione and 4β-bromo-5β-androstane-3,17-dione. Controls of 5α-androstane-3,17-dione, 5β-androstane-3,17-dione, 4-methylandrost-4-ene-3,17-dione and 4-bromoandrost-4-ene-3,17-dione were run in parallel.

Five milligrams of each substrate dissolved in 0.4 ml of dimethylformamide was incubated with 10 ml of a 20,000 x g supernatant fraction (equivalent to 1.2 g

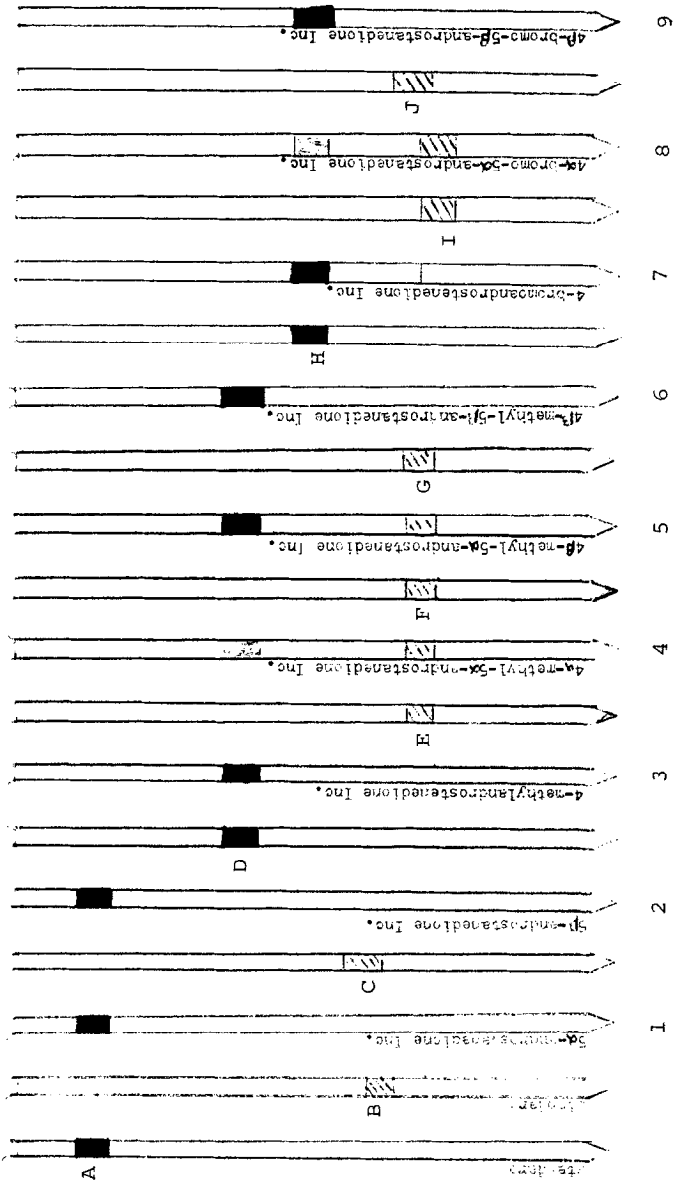

Figure 1. Paper Chromatogram Strips of Steroid Standards and Incubations with N. restrictus. All chromatograms were run for 6 hours in the toluene-propylene glycol system and the compounds were detected by Ultraviolet (UV) absorption and the Zimmermann spray reagent. Areas shaded in dark (▬) were UV absorbing and positive to the Zimmermann test while the striped areas (▨) were positive to Zimmermann test only. Steroid standards: A=androsta-1,4-diene-3,17-dione; B=5α-androstane-3,17-dione, C=5β-androstane-3,17-dione; D=4-methylandrost-4-ene-3,17-dione; E=4α-methyl-5α-androstane-3,17-dione; F=4β-methyl-5α-androstane-3,17-dione; G=4β-methyl-5β-androstane-3,17-dione; H=4-bromoandrost-4-ene-3,17-dione; I=4α-bromo-5α-androstane-3,17-dione; J=4β-bromo-5β-androstane-3,17-dione.

of wet cells) of Nocardia restrictus (ATCC 14887) and 2 mg of menadione, and the total volume brought to 15 ml with 0.03 M phosphate buffer (pH 7.0). Incubations were carried out at 28° C for three hours with continuous shaking. Shorter periods of incubation showed that the dehydrogenation of the 5β-androstane derivatives was complete in one hour while longer periods of incubation did not result in an increase in the dehydrogenation of the 5α-androstane derivatives. The incubation mixture was extracted with dichloromethane and the extent of dehydrogenation determined by measuring the optical density of the dichloromethane phase at maximum wavelength. Recovery of known amounts of steroids from these reaction mixtures was found to be constant and >95% complete. The residues from each of the dichloromethane extracts were resolved by paper chromatography using the toluene-propylene glycol system (Figure 1) and the areas absorbing in the ultraviolet were eluted from the paper strip with ethanol and their optical density measured at maximum wavelength.

Dehydrogenation of C-4 unsubstituted 5α- and 5β-androstanediones gave androsta-1,4-diene-3,17-dione (strips 1 and 2) while all substrates with a substituent at the 4 position gave only the corresponding 4-dehydrogenated product (strips 4,5,6,8 and 9). In fact, when 4-methylandrost-4-ene-3,17-dione and 4-bromoandrost-4-ene-3,17-dione were incubated with enzyme preparation, there were no transformation products formed (strips 3 and 7) indicating that the Δ^1-dehydrogenase does not act on substrates substituted in the 4 position.

The optical density value was normalized to unity for the incubation mixture extract of 4-methylandrost-4-ene-3,17-dione and 4-bromoandrost-4-ene 3,17-dione. All other values are expressed relative to that of 4-methylandrost-4-ene-3,17-dione or 4-bromoandrost-4-ene-3,17-dione. The results in Table 1 show that 4β substituted 5α-androstane-3,17-diones gave 72% of the corresponding 4-dehydro porducts while the 4α-substituted 5α-androstane-3,17-diones results in only 10-15% of the corresponding 4-dehydro products indicating that the enzymatic dehydrogenation of 5α-androstane-3,17-dione proceeds by preferential removal of the 4α and 5α hydrogens. On the other hand, 4β substituted 5β-androstane-3,17-

Table I. Optical Density Ratio of Products from N. restrictus incubations

Substrate	Product	max	Incubation mixture extract[a]	Purified Product[b]
4-methylandrost-4-ene-3,17-dione	4-methylandrost-4-ene-3,17-dione	250 mμ	1.0*	0.93
4-bromoandrost-4-ene-3,17-dione	4-bromoandrost-4-ene-3,17-dione	253	1.0+	0.90
4α-methyl-5α-androstane-3,17-dione	4-methylandrost-4-ene-3,17-dione	250	0.10	0.08
4β-methyl-5α-androstane-3,17-dione	4-methylandrost-4-ene-3,17-dione	250	0.72	0.62
4α-bromo-5α-androstane-3,17-dione	4-bromoandrost-4-ene-3,17-dione	253	0.15	0.14
4β-methyl-5βandrostane-3,17-dione	4-methylandrost-4-ene-3,17-dione	250	0.98	0.93
4β-bromo-5β-androstane-3,17-dione	4-bromoandrost-4-ene-3,17-dione	253	0.95	0.89

a. Obtained by dividing optical density values of incubation mixture extracts with optical density value of 4-methylandrost-4-ene-3,17-dione* (or 4-bromoandrost-4-ene-3,17-dione+) incubation mixture extract.

b. Obtained by dividing optical density values of purified products with optical density value of 4-methylandrost-4-ene-3,17-dione* (or 4-bromoandrost-4-ene-3,17-dione+) incubation mixture extracts.

diones yields quantitative amounts of the corresponding 4-dehydro products indicating a stereospecific removal of the 4α and 5β hydrogens.

These results indicate a cis removal of hydrogens in the 5α-series and a trans removal in the 5β-series. It is pertinent to note that the stereochemistry of enzymic dehydrogenation of 5α- and Δ^4-3-ketosteroids catalyzed by Δ^1-dehydrogenase from Bacillus sphaericus (9) N. restrictus and Septomyxa affinis (10) proceed by a trans diaxial removal of the 1α and 2β hydrogens.

The mechanism of enzymic 4,5-dehydrogenation is presently under investigation using deuterium labelled substrates of known configuration.

References

1. Vischer, E. and Wettstein, A., Adv. Enzymol. 20, 237 (1958).
2. Peterson, D.H., in C. Rainbow and A.H. Rose (Editors), Biochemistry of industrial microorganisms, Acad. press, Inc., New York, 1963, p. 537.
3. Davidson, S.J. and Talalay, P., J. Biol. Chem., 241, 906 (1966).
4. Sih, C.J. and Bennett, R.E., Biochim. Biophys. Acta, 56, 584 (1962).
5. Stefanovic, V., Hyano, M., and Dorfman, R.I., Biochim. Biophys. Acta, 71, 429 (1963).
6. Singer, T.P., Kearney, E.B., and Massey, V., Advances in Enzymol., 18, 65 (1957).
7. Beinert, H., J. Biol. Chem., 225 465 (1957).
8. Freidmann, H.C. and Vennesland, B., J. Biol. Chem., 233, 1398 (1958).
9. Ringold, H.J., Hyano, M., and Stefanovic, V., J. Biol. Chem., 238, 1960 (1963).
10. Abul-Hajj, Y.J., Ph.D. Thesis, University of Wisconsin, June 1968.