21-HYDROXYSTEROID DEHYDROGENASE A NEW ENZYME OF CORTICOSTEROID METABOLISM*

by

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An NADH dependent reduction of cortisol 21-aldehyde (Monder and White, 1961) and cortisone 21-aldehyde (Schneider, 1953) to their respective steroid 21-alcohols occurs in rat liver homogenates. We wish to report the purification of an enzyme from sheep liver which catalyzes similar NADH dependent reductions of these and other steroid 21-aldehydes. Sheep liver acetone powders were extracted with water at room temperature. The precipitate obtained between 20 and 50% saturation with ammonium sulfate (pH 7.0, 0°) was redissolved in 0.1M sodium phosphate, pH 7.4, then heated at 55° for 10 min and centrifuged at 0° to remove denaturated protein. Activity was adsorbed on alumina C_γ at pH 5.8. After washing the gel with 0.1M sodium phosphate, 7.4, the activity was extracted with the same buffer containing 10% ammonium sulfate. Further fractionation between 20 and 50% saturation ammonium sulfate (pH 7.0, 0°) yielded an enzyme that was purified 13 to 19-fold as compared to the acetone powder extract.

Cortisol 21-aldehyde was stoichiometrically reduced by the 19-fold purified enzyme as measured by cortisol formation using the blue tetrazolium reaction (Mader and Buck, 1952) and disappearance of cortisol 21-aldehyde measured as its

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p-nitrophenylhydrazone at 410 mμ. Optimum pH for reduction was 6.9 in 0.1 M phosphate and 6.2 in 0.1 M Tris-maleate buffer. K_m for cortisol 21-aldehyde was 3.9×10^{-4} M; initial velocity under a variety of conditions with respect to NADH was constant down to 10^{-5} M nucleotide, indicating that K_m for NADH is considerably below 10^{-5} M.

Enzyme was inhibited about 50% by 3×10^{-4} M solutions of hydroxymercuribenzoate, acrolein, and mercuric ions, indicating that sulfhydryl groups were involved in enzymic activity. Other metabolic inhibitors including a number of metal cations did not affect activity. Steroids found to be competitive inhibitors of the dehydrogenase were ($K_i \times 10^5$ M): androsterone (0.3), estradiol (0.32), estrone (0.42), testosterone (1.4), deoxycorticosterone (2.2), cortisol (2.7).

The relative reduction rates of a number of steroid 21-aldehydes at different stages of purification of the enzyme are shown in Table I. Initial reduction rate differed for each substrate and showed no apparent relationship to steroid structure. No appreciable changes in velocity ratios were observed during purification suggesting that a single enzyme catalyzes the reduction of the various substrates studied. Other compounds containing carbonyl groups, including acetaldehyde, glyoxal, DL-glyceraldehyde, p-tolualdehyde, o-anisaldehyde, or benzaldehyde were not reduced. Methylglyoxal was reduced at 1% of the rate of cortisol 21-aldehyde. Steroids containing carbonyl groups in positions other than at carbon 21 were not reduced by the enzyme. These included aldosterone, isoandrosterone, estrone, testosterone, cortisol, cortisone, and deoxycorticosterone.

Isolation of product: The product resulting from the reduction of cortisol 21-aldehyde was fractionated on silica gel columns using chloroform with increasing concentrations of acetone as eluant (Monder and White, 1961) and recrystallized from ethanol-cyclohexane mixtures. From 16 mg cortisol 21-aldehyde, 12 mg of a white material was isolated. This gave a spectrum in sulfuric acid (Zaffaroni, 1950) identical with cortisol (maxima: 473, 393, 280 mμ) but different from

Table I

Relative Substrate Activity During Purification of Sheep Liver

21-Hydroxysteroid Dehydrogenase

Steroid Substrate	Degree of Purification of Enzyme		
	Crude	11.5-fold	19-fold
Cortisol 21-aldehyde	1.00	1.00	1.00
Cortisone 21-aldehyde	0.58	0.67	0.69
Corticosterone 21-aldehyde	0.23	0.16	0.16
11-Deoxycortisol 21-aldehyde	1.22	1.33	1.41
Δ^1-Cortisone 21-aldehyde	0.35	0.40	0.42
9α-Fluorocortisol 21-aldehyde	-	-	1.00

Activity was measured in systems containing 1.1 μmoles steroid 21-aldehyde in 0.1 ml propylene glycol-water (1:1, v/v), 111 μg enzyme and 2.7 ml 0.1 M sodium phosphate buffer, pH 6.9, in a quartz cuvette of 10 mm light path. After 3 min preincubation in the spectrophotometer (thermostatically controlled at 30°) reaction was initiated by addition of 0.55 μmoles NADH. Absorbancy decrease was read at 340 mμ at 15 sec. intervals, and was corrected using appropriate controls containing no steroid. Recorded values indicate ratios of reduction of steroid aldehydes relative to cortisol 21-aldehyde (Δ absorbancy for cortisol 21-aldehyde = 0.340 units/min). Steroid aldehydes were synthesized by oxidation of the corresponding steroid 21-alcohols with cupric acetate in methanol (Beyler and Hoffman, 1957).

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that of cortisol 21-aldehyde (maxima: 460 (shoulder), 400, 325 (shoulder), 287 mμ). Melting point (200-212 dec.) was identical with that of cortisol. On paper chromatograms the compound moved with cortisol in two solvent systems (Bush 4B (Bush, 1952) and formamide-benzene (Zaffaroni and Burton, 1951) and the acetylated derivative moved with cortisol 21-acetate in the same systems.

Reversibility: The equilibrium of the reaction strongly favored reduction. Oxidation of cortisol could not be measured spectrophotometrically as reduction

of NAD because the propylene glycol concentration needed to keep the steroid in solution inhibited the enzyme. In another experiment, 13.9 μmoles cortisol-4-C^{14} (670,000 cpm) were incubated with 2.7 μmoles NAD and 1.66 mg 19-fold purified dehydrogenase in 0.05M phosphate buffer, pH 8.5 in 3.5 ml final volume at 24° for 3 hr. An ethyl acetate extract of the incubation mixture was chromatographed on filter paper sheets using Bush system 4B. The radioactive areas corresponding to cortisol 21-aldehyde (250 cpm) and cortisol (18,000 cpm) were eluted and portions rechromatographed with authentic carrier, with which they migrated. The isolated cortisol 21-aldehyde had in sulfuric acid a spectrum identical with that of authentic cortisol 21-aldehyde treated in the same manner. Calculated equilibrium constant on the basis of the above data is 3.2×10^{13}, favoring reduction.

It is concluded that the enzyme isolated from sheep liver catalyzes the reversible dehydrogenation of the alcohol group at position 21 of cortisol and probably other corticosteroids. Because its specificity is apparently restricted to corticosteroids, the enzyme has been named 21-hydroxysteroid dehydrogenase. The significance of this enzyme in corticosteroid metabolism remains to be explored.

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