Reduction of the Non-Steroid Adamantanone by Crystalline Preparations of Cortisone Reductase

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Cortisone reductase (EC 1.1.1.53) has 20β -hydroxy steroid-NAD⁺ oxidoreductase activity (Hübener & Lehmann, 1958) and 3-hydroxy steroid-NAD⁺ oxidoreductase activity towards certain 5α - and 5β -androstane steroids (Gibb & Jeffery, 1970). The two activities appeared not to be independent kinetically, and this enzyme may possibly contain one active centre with complex steroid-binding characteristics (Gibb & Jeffery, 1971).

To determine whether the non-steroid adamantanone (Fig. 1) was a substrate, it was incubated (at 25°C at pH 7.0) with enzyme (7.4 µg/ml) and NADH (150 µM) in 0.1M-sodium phosphate buffer containing 1.43% (v/v) of dimethyl sulphoxide. Oxidation of NADH was followed spectrophotometrically at 340nm in a Unicam SP.800 double-beam spectrophotometer. The reference cell contained the same ingredients as the reaction cell, but no adamantanone, and only 120 µM-NADH. Addition of adamantanone (as a solution in dimethyl sulphoxide) to the other ingredients in the reaction cell resulted in oxidation of NADH, and the initial rates of reaction were determined in quadruplicate at each of six concentrations of adamantanone in the range 500–1300 µM. Careful observation by eye and by light-scattering indicated that the adamantanone remained in solution under these conditions. Regression calculations of the reciprocal of the initial reaction rate on the reciprocal of the substrate concentration gave a correlation coefficient of 0.96,

unweighted K_m 15.5 mM, unweighted V_{max} 11.7 µmol/min per mg, weighted K_m 13.2 (S.E.M. $\pm$ 10.6) mM and weighted V_{max} 10.3 (S.E.M. $\pm$ 7.9) µmol/min per mg (by using the weighting procedure of Wilkinson, 1961). These estimates of the kinetic constants were uncertain because the maximum substrate concentration that could be used was small compared with the apparent K_m value.

By using the same spectrophotometric technique it was shown that addition of adamantan-2-ol (250 µM) to the enzyme (55.5 µg/ml) and NAD⁺ (2 mM) resulted in the formation of NADH. Pregn-4-ene-3,20-dione (20 µM) lowered the rate of oxidation of adamantan-2-ol (250 µM) by more than 50% (from about 0.035 to about 0.014 µmol/min per mg).

Adamantanone is a rigid structure (Fig. 1) resembling the A-ring of both 5α - and 5β -steroids (Ringold *et al.*, 1967). The finding that it is a substrate for this enzyme is consistent with the view that the active centre is larger than a steroid molecule, and that it is probably hydrophobic in the region where the A-ring of a steroid lies during reaction at the 3-position.

Pregn-4-ene-3,20-dione is a substrate for the 20β -hydroxy steroid-NAD⁺ oxidoreductase activity of the enzyme under reducing conditions (the K_m is about 1 µM; White & Jeffery, 1972). The finding that pregn-4-ene-3,20-dione inhibited the oxidation of adamantan-2-ol indicated that reaction of this non-steroidal 3-hydroxy steroid analogue was not independent of the 20β -hydroxy steroid-NAD⁺ oxidoreductase activity of the enzyme. Thus, if more than one active centre is involved in these reactions, there is evidently interaction between the centres; perhaps a simpler concept is of one active centre with complex substrate-binding properties.

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Fig. 1. Formula for adamantanone

The numbers shown indicate the analogous ring-A atoms of 5α - and 5β -steroids. The numbering for the adamantane ring system (not shown) would make this the 2-oxo compound. There is one adamantan-2-ol, which is equally analogous to the 3α -ol and the 3β -ol of a 5α - or a 5β -steroid.

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