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DEHYDROEPIANDROSTERONE IS A SUBSTRATE FOR ESTRADIOL 17 β-DEHYDROGENASE FROM HUMAN PLACENTA

G. Mendoza-Hernández, M. Calcagno, H.R. Sánchez-Nuncio and J. C. Díaz-Zagoya

Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, Apartado Postal 70-159 04510 México, D. F. MEXICO

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Summary: The enzyme estradiol 17 β-dehydrogenase (17 β-ED) [E.C.1.1.1.62] from human placenta was purified to homogeneity by the initial steps of a published procedure, followed by an affinity chromatography step in Reactive Blue 2-Sepharose, eluting with NADP. The pure enzyme is not specific for estrogenic substrates, it also catalyzes the oxidation-reduction of several androgens and progesterone (i.e. dehydroepiandrosterone, androstenedione, 5 α-dihydrotestosterone, and 20 α-dihydroprogesterone). The comparison of the kinetic parameters for these substrates, shows that dehydroepiandrosterone could be a physiological ligand of the enzyme, and consequently involved in the control of its function in estrogen metabolism.

Estradiol 17 β-dehydrogenase (17 β-ED) [E.C.1.1.1.62] from human term placenta was first described by Langer and Engel (1). This enzyme catalyzes the reversible interconversion of estradiol 17-β and estrone utilizing both NADP and NAD as coenzyme. This dehydrogenase has been described as a specific oxidoreductase for estrogen substrates with absolute stereospecificity for the 17-β configuration of hydroxyl group in ring D and with requirement for an aromatic A ring, B ring or both (2). The enzyme was purified to apparent homogeneity by ion exchange chromatography (3), and later by an estrogen-bound affinity chromatography (4). The 20α-hydroxysteroid dehydrogenase (20α-HSD) [E.C.1.1.1.149] was described and characterized in human placenta by Zander et al. as an enzyme, that catalyzes the conversion of 20 α-dihydro-progesterone (20α-hydroxypreg-4-en-3-one; 20α-DHP) to progesterone (5). The 20α-HSD and 17α-ED activities are non-separable. Purdy et al. first suggested

Abbreviations used: 17 β-ED,- estradiol 17β-dehydrogenase; 20α-HSD,- 20α-hydroxysteroid dehydrogenase; 20α-DHP,- 20α-dihydroprogesterone; DHA,- dehydroepiandrosterone; DHA-S, dehydroepiandrosterone sulfate.

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that both activities are located in the same protein (6). The double activity hypothesis is supported by Strickler et al. (7). This group purified a single enzyme with a constant activity ratio for both substrates over all the purification procedure.

In the present study we report that the estradiol 17-β dehydrogenase from human term placenta, purified to homogeneity not only catalyzes the oxidation-reduction of estrogens and progesterone but also utilizes dehydroepiandrosterone (DHA) and other androgens as substrates. Our results show that in human placenta a single enzyme catalyzes the oxidation-reduction of estrogens, androgens and progesterone and supports the possible role of adrenal androgens, particularly DHA in the modulation of estrogen metabolism.

MATERIALS AND METHODS

Reagents. Steroids were purchased from Sigma Chemical Co. (St. Louis, Mo., USA) and repurified by thin layer chromatography before use. NADPH and proteins used as standard were obtained also from Sigma. DEAE-Sephacel and Sepharose were products from Pharmacia Fine Chemicals (Uppsala, Sweden). Reactive Blue 2 (Cibachrome Blue 3FG-A) was a gift from Ciba-Geigy Mexicana, S.A. de C.V. Other reagents were of analytical grade.

Enzyme purification. The enzyme was prepared from human term placenta, combining the first five steps of the scheme reported by Jarabak (3), with an affinity chromatography step in Reactive Blue 2-Sepharose (Blue Sepharose). The previous steps involved homogeneization, ammonium sulfate fractionation, heat treatment, a second ammonium sulfate fractionation and a DEAE - Sephacel chromatography. The partially purified enzyme from ion exchange chromatography (specific activity 0.35 units/mg of protein) was dialyzed against 5 mM potassium phosphate buffer containing 1 mM EDTA and 10 per cent glycerol, and applied into 1.6 × 15 cm column of Blue Sepharose. This affinity matrix, was prepared coupling the dye to the gel beads, according to Böhme (8). The column was washed with the buffer, and the enzyme was eluted with 0.25 mM NADP dissolved in the same buffer. The enzyme appears as a single peak of constant specific activity. The fractions containing the enzyme were pooled, concentrated by ultrafiltration (Amicon Diaflo ultrafilter PM-10) and dialyzed against the same buffer, but containing 50 per cent glycerol.

Enzyme assays. All enzyme assays and kinetic studies were made measuring spectrophotometrically the reduction of NADP or the oxidation of NADPH, at 340 nm, in a single beam automatic recording spectrophotometer, (Gilford 252) or a double beam recording spectrophotometer (Pye Unicam SP-1800) at 37°C. The latter was preferred to record the disappearence of the NADPH, to correct for the spontaneous oxidation of the coenzyme at pH values lower than 7.5. Incubation mixtures were prepared in 500 µl cells and contained 5 mM potassium phosphate buffer (pH 7.0) containing 20 per cent glycerol, 10 µM NADP (or 0.6 µM of NADPH for the reverse reactions) and variable amounts of substrates, usually in the range between 0.25 to 5 Km. Reactions were started adding the enzyme. One enzyme unit is defined as the amount of enzyme that catalyzes the conversion of 1 µmole of substrate per minute, at pH 7.0 and 37°C.

Protein determinations were made by the Coomassie blue G-binding method of Bradford (9) with chicken egg albumin as reference. This protein was standarized spectrophotometrically by means of its absortivity at 280 nm (10).

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was made using the discontinuous buffer system of Laemmli (11), and stained by standard methods.

Computer aided statistical analyses of kinetic data were made according to Cleland (12) using a TRS-80 Radio Shack microcomputer, programmed in BASIC language. This program computes Km and Vmax with their standard errors and gives the corrected curve from hyperbolic kinetic data.

RESULTS AND DISCUSSION

The final product obtained by the purification procedure described, possessed a specific activity for 17 β-ED of 7.1 units/mg. This activity agrees with that reported by Chin et al. for the crystallized enzyme (13). The protein displayed a single sharp band on sodium dodecyl sulfate disc-gel-electrophoresis; the monomer molecular weight calculated by means of this procedure was 35 000, which corresponds to that reported for the estradiol 17 β-ED subunit (14). The enzyme possesses both 17 β-ED and 20α-HSD activities and the ratio of specific activities for their respective substrates was approximately 100:1 as reported by Strickler et al. (7).

The enzyme not only catalyzed oxidation-reduction of estradiol 17-β and progesterone but also catalyzed the oxidation-reduction of testosterone, 5α-dihydrotestosterone and dehydroepiandrosterone sulfate (DHA-S) or alcohol. The rates of oxidation-reduction of these androgens were about 2-12 times higher than the rate of oxidation of 20αDHP. For the androgens tested the enzyme was particularly active for the reduction of DHA, as shown in Fig. 1. The kinetic parameters found for human placental 17 β-ED with different substrates are summarized in table I. The Km for the oxidation of 17-β was 7×10^{-6} M which is in close agreement with the value reported by other investigators (15). On the other hand, the Km for the reduction of DHA was nearly 60×10^{-6} M, and the maximal velocity per mg of enzyme about 1 mole/min; these values are the same for both the free steroid and its sulfate. Although the Km for DHA is 8.6 times higher than the Km for estradiol 17-β, it is necessary to take into account the concentrations of these steroids under physiological conditions. Mathur et al. measured the concentrations of DHA + DHA-S and estradiol 17-β in the plasma of pregnant women, finding

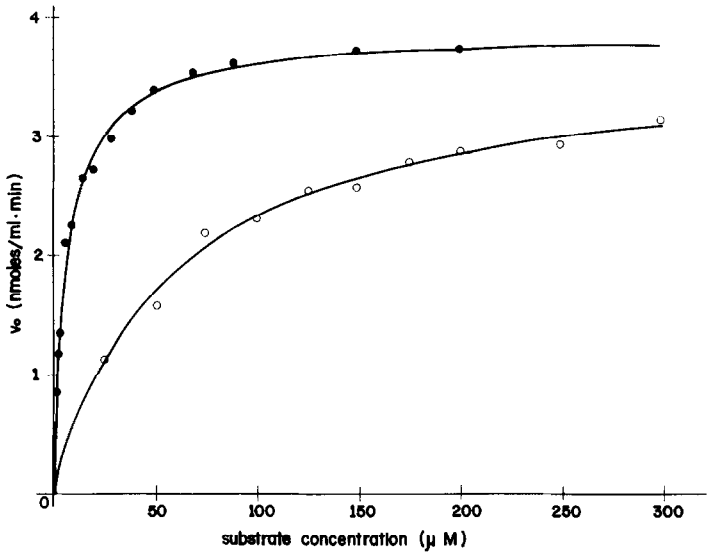

Fig. 1. Direct plot of initial velocities as a function of steroid substrate concentration. Estradiol 17-β (● - ●); dehydroepiandrosterone (o - o). Enzyme concentration for estradiol 17-β was 5.3 μgml^{-1} and for dehydroepiandrosterone 39.75 μgml^{-1}. The conditions are described in the text. Full lines are corrected curves given by the computer; kinetic parameters are shown in table I.

813 ± 60 ng/ml (2.1 μmoles/l) and 16.8 ± 1.3 ng/ml (0.06 μmoles/l) respectively, at term, before the onset of labor (16).

According to our kinetic data, and the steroid levels reported, DHA and its sulfate, could be considered important alternative substrates or product inhibitors of this enzyme, depending upon the NADP/NADPH ratio in the cells. Though the physiological role of 17 β-ED has not been established, the

TABLE I
Kinetic parameters found for human placental estradiol 17β-dehydrogenase with different substrates

Substrate (variable)	coenzyme (constant)	Km ± SE ($M \times 10^{-6}$)	Specific activity ± SE (μmoles/min mg of protein)
Estradiol 17-β	NADP	7.0 ± 0.6	7.1 ± 0.13
Dehydroepiandrosterone	NADPH	59.8 ± 5.4	0.92 ± 0.03
5α-dihydrotestosterone	NADP	118.0 ± 5.0	0.27 ± 0.01
Androstenedione	NADPH	124.0 ± 14.0	0.17 ± 0.01
Testosterone	NADP	263.0 ± 33.0	0.20 ± 0.02
20α-dihydroprogesterone	NADP	207.0 ± 17.0	0.078 ± 0.004

perfusion experiments carried out by Tseng et al. (17) pointed that the direction of the reaction in whole placenta is from estradiol 17-β to estrone; then it would be expected that DHA would act under physiological conditions as an alternative product inhibitor. Recently, Bonney et al. (18) reported a strong inhibition of 17 β-ED activity in human endometrial homogenates by DHA and DHA-S, using a radioisotope conversion assay. This method to measure the enzyme activity does not allow, the detection of the possible function as substrates, of the steroids assayed, as inhibitors. Then, these observations could be interpreted as inhibition by an alternative product, as predicted by our results.

The present study shows that in human placenta, a single enzyme catalyzes oxidation reduction reactions with estrogens, androgens and progesterone, and supports the hypothesis that adrenal androgens could have a modulation role on estrogen metabolism.

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REFERENCES

1. Langer, L.J. and Engel, L.L. (1958) J. Biol. Chem. 233: 583-588
2. Karabolas, H.J. and Engel, L.L. (1971) Endocrinol. 88: 1165-1169
3. Jarabak, J. (1969) Methods in Enzymol 15: 746-752
4. Chin, C.C. and Warren, J.C. (1973) Steroids 22: 373-378
5. Zander, J., Forbes, T.T., Van Munstermann, A.M. and Neher, R.J. (1959) Clin. Endocrinol. Metab. 18: 337-353
6. Purdy, R.H., Halla, M. and Little, B., (1964) Biochim. Biophys. Acta. 89: 557-560
7. Strickler, R.C. and B. Tobias (1980) Steroids 36: 243-253
8. Böhme, H.J., Kopperschalager, G., Schulz, J. and Hofmann, E.J. (1972) Chromatography Anal 69: 209-214
9. Bradford, M.M. (1976) Anal. Biochem 72: 248-254
10. Kirschenbaum, D.E. (1973) Anal. Biochem. 56, 237-263
11. Laemmli, U.K. (1970) Nature 227: 680-685
12. Cleland, W.W. (1979) Methods in Enzymol 63: 103-138 Academic Press
13. Chin, C.C., Dence, J.B. and Warren, J.C. (1976) J. Biol. Chem. 256: 316-321
14. Burns, D.J., Engel, L.L. and Bethune, J.L. (1971) Biochem. Biophys. Res. Comm. 44: 786-792
15. Brian, T., Covey, D.F. and Strickler, R.C. (1982) J. Biol. Chem. 257: 2783-2786
16. Mathur, R.S., Land Grebes, Moody, O.L. Powell, S. and Williamson, H.O. (1980) J. Clin. Endocrinol Metab. 51, No. 6 1235-1238
17. Tseng, L., Stollee, A. and Gurpide, E. (1972) Endocrinology 90: 405-414
18. Bonney, R.C., Reed, M.J. and James, V.H. (1983) J. Steroid Biochem. 18: 59-64.