J. steroid Biochem. Vol. 23, No. 6A, pp. 1079–1081, 1985
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0022-4731/85 $3.00 + 0.00
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SHORT COMMUNICATION

KINETIC STUDIES OF OESTRADIOL 17β-HYDROXYSTEROID DEHYDROGENASE IN MCF-7 MAMMARY CANCER CELLS

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(*Received* 3 *June* 1985)

Summary—The kinetic properties of oestradiol 17β-dehydrogenase have been studied in the MCF-7 human breast cancer cell line. The activity of the enzyme was found to be linear with respect to time for at least 2 h and with respect to protein concentrations over the range 40–300 μg protein per tube. The enzyme was able to utilize both NAD and NADP as cofactors but at higher concentrations NAD was the more effective. The apparent K_m was estimated to be 13.4 μM which is approximately two-fold higher than found for breast tumour tissue.

INTRODUCTION

Current understanding of the hormonal regulation of human breast cancer has been aided substantially by information obtained from the study of MCF-7 cells. These cells contain receptors for oestrogens and are biologically responsive to oestradiol [1, 2]. Oestrone, however, is considered to be less potent and to exert its effect through metabolism to oestradiol [3].

Mammary tumour tissue has the capacity to oxidize oestradiol to oestrone by the action of 17β-hydroxysteroid dehydrogenase (17OHSD) [4] but the activity of this enzyme with respect to oestradiol oxidation in MCF-7 cells has not been investigated. In view of the importance attached to the use of MCF-7 cells as a model for human breast cancer studies, we report here on the kinetic properties of 17OHSD in this cell line.

EXPERIMENTAL

Steroids

[4-^{14}C]Oestrone (SA = 55.8 mCi/mmol) and [2,4,6,7-^{3}H]-oestradiol (SA = 98 Ci/mmol) were purchased from Amersham International plc. Purity was checked before use in a Bush B3 chromatography system. Unlabelled oestradiol was obtained from the Sigma Chemical Co. (Poole, Dorset, U.K.).

MCF-7 cells

The MCF-7 human breast cancer cell line was obtained from the Royal Marsden Hospital, London. The cells were grown as a monolayer in plastic flasks in Dulbecco's modified Eagles medium supplemented with 10% foetal calf serum in 10 μg/ml insulin. They were harvested using 0.05% trypsin and 0.02% EDTA, washed three times with culture medium and resuspended in 0.1 M phosphate buffer pH 7.4. The cell suspension was stored at −20°C and homogenized using a Polytron homogenizer immediately before assay.

Kinetic studies

Kinetic studies were carried out using methods described elsewhere [4]. Briefly, aliquots of MCF-7 cell homogenate were incubated at 37°C in 10 × 1.5 cm disposable glass tubes in 0.1 M phosphate buffer, pH 7.4. The tubes contained [^{3}H]oestradiol (10^6 cpm) and unlabelled oestradiol at the required concentration together with NAD, in a final volume of 350 μl. Procedural losses were monitored by the addition of [^{14}C]oestrone. Steroids were dispensed in ethanol and evaporated to dryness before the addition of buffer. The reaction was initiated by the addition of NAD and terminated with 3 ml diethyl ether which was subsequently used for extraction. Control tubes were processed with each assay. Oestrone was separated from oestradiol by thin-layer chromatography and radioactivity measured as described previously [4, 5]. Protein was measured by the method of Lowry *et al.*[6] and results were expressed where applicable as pmol oestrone produced/mg protein per min.

Optimum incubation time, enzyme concentration and NAD concentration were determined by varying the time of incubation and the amount of enzyme or cofactor added. The apparent Michaelis–Menton constant (K_m) was determined by the method of Lineweaver and Burk [7] using duplicate measurements of initial velocity over a range of substrate concentrations at a single time point of 18 min.

RESULTS

Enzyme characterization studies

Incubation time. The time-course of the conversion of oestradiol to oestrone by 17OHSD in MCF-7 cells is shown in Fig. 1. The formation of oestrone from oestradiol was found to be linear with time for a least 120 min.

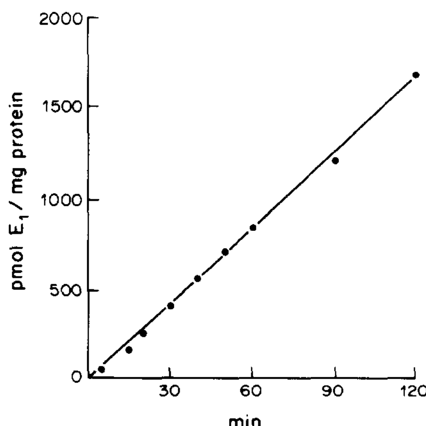

Fig. 1. Time-course of the conversion of oestradiol to oestrone by oestradiol 17β-hydroxysteroid dehydrogenase in MCF-7 cells.

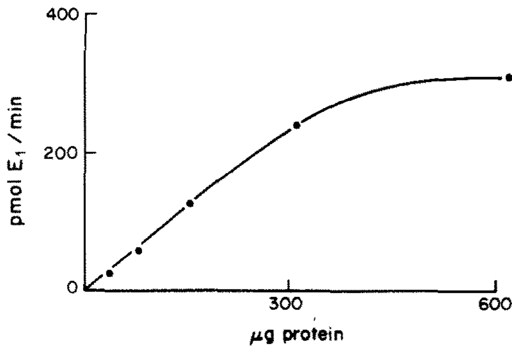

Fig. 2. The effect of enzyme concentration on the conversion of oestradiol to oestrone in MCF-7 cells.

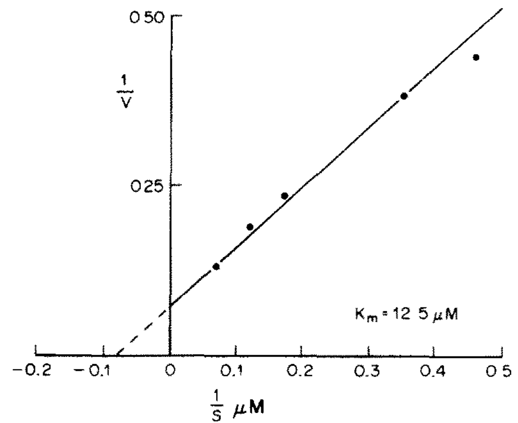

Fig. 4. Lineweaver–Burk plot of initial velocities of oestradiol oxidation at various concentrations of substrate. Velocity is expressed as pmol oestrone produced/mg protein per min.

Enzyme concentration. The effect of enzyme concentration on the conversion of oestradiol to oestrone in MCF-7 cells is demonstrated in Fig. 2. A linear relationship between the amount of enzyme and the formation of product was found over the range 40–300 μg protein/tube.

Cofactor concentration. Figure 3 depicts the conversion of oestradiol to oestrone by MCF-7 cells as a function of cofactor concentration. The enzyme was able to utilize both NAD and NADP as cofactors and at low concentrations it is evident that both are equally effective. However, at higher concentrations, the maximum activity in the presence of NADP was only 64% of that achieved in the presence of NAD. V_{max}-values calculated from a Lineweaver–Burk plot were 10 and 5 pmol oestrone formed/mg protein per min for NAD and NADP, respectively.

Apparent Michaelis–Menton constant. The initial velocity of 17OHSD in MCF-7 cells was determined at concentrations of oestradiol between 2.8 and 28 μM. A typical Lineweaver–Burk plot is shown in Fig. 4. The K_m-value was estimated in three separate experiments and the values obtained were 13.33, 12.50 and 14.28 μM (mean value, 13.37 μM).

DISCUSSION

Our study has demonstrated the presence of oestradiol 17β-dehydrogenase in MCF-7 mammary cancer cells. Comparison of the kinetic properties of this enzyme with those of the breast tumour enzyme described in an earlier study [4] reveals a difference in the apparent K_m-value. We reported a K_m of 2.64 μM for the breast tumour enzyme which is similar to the value found for the enzyme in human endometrium [5, 8]. The K_m calculated here for MCF-7 cells is five-fold higher than in these tissues which suggests that these cells have a reduced ability to oxidize oestradiol. Support for this observation comes from the work of Rademaker *et al.*[9] who reported that the conversion of oestradiol to oestrone by MCF-7 cells after 48 h in culture was only 7%. However, with respect to cofactor requirements, both the tumour enzyme [4] and the MCF-7 cell enzyme were able to utilize NAD and NADP although in each instance NAD was the preferred cofactor.

The presence in MCF-7 cells of a 17OHSD responsible for the metabolism of testosterone to androstenedione has been reported by MacIndoe and Woods [10]. The enzyme showed a requirement for the cofactor NADP but the potency of NAD was not tested. Further studies would be required to determine whether the enzyme described by these workers is similar to the oestradiol dehydrogenase investigated in the present study.

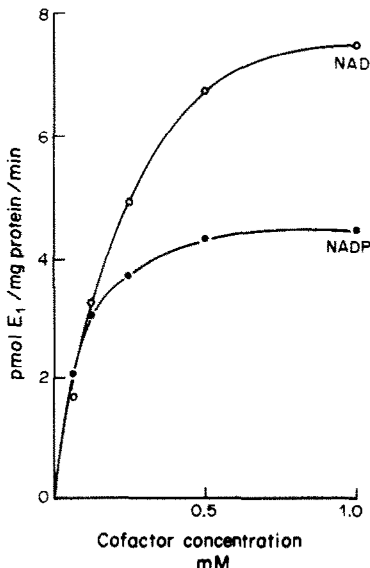

Fig. 3. The conversion of oestradiol to oestrone as a function of cofactor concentration.

Acknowledgement—This work was supported by a grant from the Cancer Research Campaign.

REFERENCES

1. Lippman M., Bolan G. and Huff K.: The effects of estrogens and antiestrogens on hormone responsive human breast cancer in long term tissue culture. *Cancer Res.* **36** (1976) 4595–4601.
2. Lippman M., Monaco M. E. and Bolan G.: Effects of estrone, estradiol and estriol on hormone responsive human breast cancer in long-term tissue culture. *Cancer Res.* **37** (1977) 1901–1907.
3. MacIndoe J. H., Woods G. R. and Etre L. A.: The specific binding of estradiol and estrone and the subsequent distribution of estrogen–receptor complexes within MCF-7 human breast cancer cells. *Steroids* **39** (1982) 245–258.
4. Bonney R. C., Reed M. J., Davidson K., Beranek P. A. and James V. H. T.: The relationship between 17β-hydroxysteroid dehydrogenase activity and oestrogen concentrations in human breast tumours and in normal breast tissue. *Clin. Endocr.* **19** (1983) 727–739.

5. Bonney R. C., Reed M. J. and James V. H. T.: Inhibition of 17β-hydroxysteroid dehydrogenase activity in human endometrium by adrenal androgens. *J. steroid Biochem.* **18** (1983) 59–64.
6. Lowry O. H., Rosenbrough N. J., Farr A. L. and Randall R. J.: Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193** (1951) 265–275.
7. Lineweaver H. and Burk D.: The determination of enzyme dissociation constants. *J. Am. chem. Soc.* **56** (1934) 658–666.
8. Tseng L., Mazella J. and Tseng L.: Kinetic studies of human endometrial hydroxysteroid dehydrogenase. *J. steroid Biochem.* **14** (1981) 437–442.
9. Rademaker B., Vossenberg J. B. J., Poortman J. and Thijssen J. H. H.: Metabolism of estradiol-17β, 5-androstene-3β,17β-diol and testosterone in human breast cancer cells in long-term culture. *J. steroid Biochem.* **13** (1980) 787–791.
10. MacIndoe J. H. and Woods G. R.: Steroid metabolizing enzymes in human breast cancer cells, II. 5α-reductase, 3β-hydroxysteroid oxidoreductase and 17β-hydroxysteroid oxidoreductase. *Endocrinology* **108** (1981) 1407–1413.