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THE ACTIVITY OF MOUSE UTERINE ACID PHOSPHATASE ON OESTRADIOL-3- AND OESTRADIOL-17β-PHOSPHATE

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SUMMARY

Oestradiol-3- or 17-phosphates are substrates for the acid phosphatase of mouse uterus. Using these conjugates a double pH optimum is obtained at pH 4·5 and 6·5, which does not seem to be related to the presence of isoenzymes with different pH optima. Gel filtration on Sephadex G200 resolved the enzyme into two fractions, both active on these substrates at pH 4·5 and 6·5.

Study of the distribution of acid phosphatase activity after subcellular fractionation showed that at both pH optima the activity is detectable in the mitochondrial-lysosomal fraction and in the 20,000 × g supernatant. The lysosomal activity was released by Triton X100 treatment.

It appears that the double pH optimum is not linked to a subcellular distribution of acid phosphatase variants with different pH optima. The enzyme has a dual subcellular localization, lysosomal and non-lysosomal.

INTRODUCTION

UTERINE acid phosphatase (E.C. 3.1.3.2, orthophosphoric monoester phosphohydrolase) obtained from adult mice hydrolyzes 17-oestradiol-3- or 17-phosphates [1]. Since the mechanism of action of oestrogens on the uterus is not yet well known, it seems of interest to study all aspects of oestrogen metabolism. In this regard the study of the action of phosphatases on oestrogen phosphates might contribute to our understanding of the mechanism of action of steroid hormones. These compounds have been found to be synthesized *in vitro* by rat liver[2,3] and by liver, ovary and uterus of rabbit[4]. This report studies the action of uterine phosphatase on oestradiol-3- and 17-phosphates.

EXPERIMENTAL

Adult mice of BALB/C strain were used for this work. The animals were killed by cervical dislocation; the uteri were quickly dissected out and after removal of fat tissue, were pooled together regardless of the stage of the sexual cycle. Pooled uteri from 20–40 animals were homogenized in 15 ml of 0·25 *M* sucrose, containing 5 m*M* 2-mercaptoethanol and 1 m*M* EDTA, unless otherwise indicated. After centrifugation at 900 × g for 30 min at 4°C in a Sorvall refrigerated centrifuge the supernatant was used directly for enzymatic assays.

The evaluation of uterine acid phosphatase activity as a function of pH using NPP* or oestradiol phosphates as substrate, was carried out as follows. The incubation medium contained 2 ml of 0·1 *M* buffer (glycine-HCl pH 3 and 3·5; acetic acid-sodium acetate pH 4 and 4·5; tris-HCl pH 5 to 7), 360 nmoles of NPP and 100 μl of enzyme solution. The incubation was carried out for 30 min at 37°C and was stopped by adding 0·5 ml of 0·1 *M* NaOH. The NP formed was estimated at 400 mμ in

*NPP = p-nitrophenyl phosphate; NP = nitrophenol.

a Beckman spectrophotometer. When oestradiol phosphates were used as substrates 86 nmoles of oestradiol-3- or 17-phosphates was added to the incubation medium together with 0·5 ml of the enzyme solution. The reaction was carried out at 37°C for 30 min and stopped by adding 0·5 ml of 0·1 *M* NaOH. The extraction and quantitative analysis of oestradiol was performed by our previous method [1].

For fractionation of the acid phosphatase the 900 × g supernatant was directly applied on a Sephadex G200 column (3·5 × 36 cm) previously equilibrated at 4°C with 0·01 *M* tris-HCl buffer, pH 7·4. The same buffer was used for sample elution; fractions of 30 drops each were collected.

For subcellular localization of the acid phosphatases the uterine tissue (2–3 g) was homogenized for several minutes in 15 ml of 0·25 *M* sucrose at a very low speed in a teflon homogenizer. The homogenate was filtered first through two layers of sterile gauze and then through a thin layer of glass wool. The filtered homogenate was centrifuged at 900 × g for 10 min. The sediment was resuspended in the original volume of 0·25 *M* sucrose and centrifuged at 900 × g and the procedure repeated, to obtain the nuclear fraction. The supernatant solution was recentrifuged at 20,000 × g for 30 min and the new sediment washed twice, resuspended in the original volume of 0·25 *M* sucrose and designated as mitochondrial-lysosomal fraction. The 20,000 × g supernatant was designated as supernatant fraction.

The protein content was estimated by the method of Lowry *et al.*[5] using bovine serum albumin as standard.

Oestradiol-3-phosphate was generously donated by Dr. D. L. DiPietro, Dept. of Obstetrics and Gynecology, School of Medicine, Vanderbilt University, Nashville, Tennessee. Oestradiol-17-phosphate was purchased from Mann Research Laboratories, New York. Chemicals for gas-chromatographic analysis of oestradiol were obtained from Applied Science Laboratories, State College, Pennsylvania. In all cases redistilled solvents were used. Silica gel plates without fluorescent indicator were supplied by Merck, A.-G., Darmstadt, Germany.

RESULTS

The activity of uterine acid phosphatase as a function of pH using NPP or oestradiol-17-phosphate as substrate is shown in Fig. 1. Using NPP the optimum activity was in the range pH 4–5 whereas with oestradiol-3 or 17-phosphates two pH optima were observed, the first at pH 4·5 and the second, less active, at pH 6·5. No significant difference was found in the enzyme activity between the two steroid phosphates as substrates.

The double pH optimum with steroid phosphates may imply the presence of two variants of the uterine acid phosphatase acting upon these substrates at

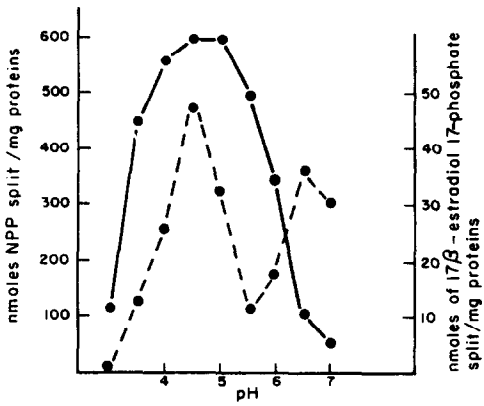

Fig. 1. Uterine acic phosphatase activity as function of pH using NPP (——) or oestradiol-3- or 17-phosphates (– – –) as substrates. Each point is the average of four observations. The incubation medium contained 1·2–1·8 mg of proteins.

different pH optima. To verify this hypothesis in several experiments acid phosphatase was fractionated on a Sephadex G200 column. In each experiment 8 ml of the 900 × g supernatant from 2–3 g of uterine tissue was used. The results of gel-filtration are indicated in Fig. 2. With NPP as substrate the acid phosphatase was consistently resolved into three peaks of activity, the first after the void volume, and the third always less active than the first and second. With oestradiol-3- or 17-phosphates the enzymatic activity at pH 4·5 and 6·5 coincided with the first two peaks, seen with NPP as substrate. Attempts to examine the homogeneity of these fractions by starch gel electrophoresis were unsuccessful, because of the difficulty of demonstrating directly on gel strips the phosphatase activity using steroid phosphates as substrates.

The double pH optimum could also be related to a different subcellular localization of acid phosphatase variants. This hypothesis was verified by studying the enzymatic activity at pH 4·5 and 6·5 of the nuclear, mitochondrial-lysosomal and supernatant fractions of the uterine tissue. The results are summarized in Table 1, and indicate that at both pH values more than 60 per cent of the enzymatic activity is localized in the 20·000 × g supernatant. The mitochondrial-lysosomal fraction contained some 30 per cent of the activity which was released after incubation with Triton X100 (0·5 per cent).

DISCUSSION

Oestradiol-3- and 17-phosphates are substrates for the acid phosphatase of mouse uterus. Although the rate of hydrolysis of these substrates is low, the activity is demonstrated consistently. The pH curve shows a double pH optimum at pH 4·5 and 6·5. Ma and Chan[6], using NPP as substrate, recorded in human placental acid phosphatase a double pH optimum at pH 5·2 and 5·5, suggesting

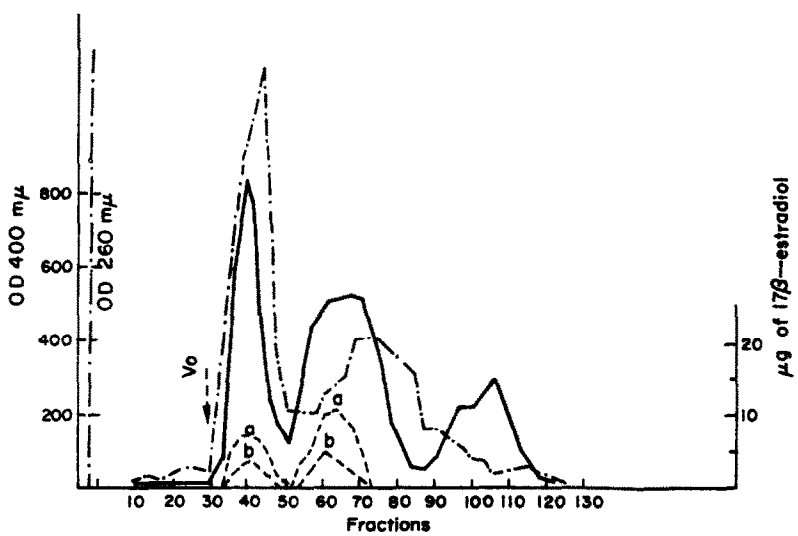

Fig. 2. Elution pattern of uterine acid phosphatase from Sephadex G200 column. (——) acid phosphatase at pH 5 using NPP as substrate (0·1 ml of each fraction was used in the incubation medium). (– – –a), (– – –b) activity with oestradiol-3- or 17-phosphate at pH 4·5 and 6·5 respectively (0·5 ml of each fraction was incubated). (· – · – ·) protein absorbancy at 260 mμ. Vo = void volume.

Table 1. Acid phosphatase activity of subcellular fractions of mouse uterus on oestradiol-17-phosphate

Cellular fractions*	Activity (nmoles/mg of proteins after 30 min) pH 4·5	pH 6·5
Nuclear	0	0
Mitochondrial-lysosomal	*t*‡	*t*
Mitochondrial-lysosomal + Triton X100†	11	7
Supernatant	26	18

*Assay systems with supernatant, mitochondrial-lysosomal and nuclear fractions contained 2·9, 1·9 and 0·5 mg of proteins respectively.

†Triton X100 was added to a final conc. of 0·5 per cent and incubated for 30 min at 0°C before the enzymatic assay.

‡*t* = below 1 μg.

the presence of more than one acid phosphatase in this organ. Moreover, several other enzymes show a double pH optimum and this is interpreted as due to the presence of different enzyme variants with different pH optima or as the expression of a particular kinetic behaviour (cf. [7]).

The behaviour of uterine acid phosphatase (Fig. 2) with oestradiol-3- or 17-phosphate seems to indicate that in the pool of acid phosphatase specific variants for steroid phosphates are not present. In human placenta three different isoenzymes of acid phosphatase were obtained by gel filtration on Sephadex G200 [8]; the last peak (III) shows a remarkable preference for oestradiol-3-phosphate [9]. This result differs from our observations upon mouse uterine phosphatase.

The double pH optimum does not seem to be related to the enzymatic variants with different subcellular localization. A dual localization of acid phosphatase in the lysosomal and microsomal fractions seems to be indicated by our result. In rat liver two acid phosphatases were obtained from lysosomes and supernatant respectively[10]. Similar results were reported for acid hydrolases obtained from tissues of different animal species[11–13]. Recent cytochemical and biochemical studies have localized the acid phosphatase activity in the endoplasmic reticulum and in lysosomes[14–17].

The question arises whether the steroid phosphates are natural substrates for acid phosphatases. In fact the presence of these conjugates *in vivo* is uncertain. Only a few reports on the presence of dehydroepiandrosterone associated to phosphate groups in human plasma are available[18–20]. Moreover Brooks *et al.* [2, 3] have shown that on incubating liver slices *in vitro* with oestrogens, some phosphate conjugates are formed. Botte *et al.*[4] found formation of estrogen phosphates on incubating *in vitro* with estrogens slices of liver, ovary and uterus of adult rabbits. An inhibition of the level of amino-transferase activity by oestrogen phosphates has been shown in rat heart[21].

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