In Vitro Inhibition of Alkaline Phosphatase by Estrogenic Hormones

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With regard to the mode of action of estrogenic substances it seemed to be of great interest to study the interaction between estrogenic hormones and alkaline phosphatase. Data available in the literature on diminished phosphatase activity in different organs [1] after the administration of sex hormones do not clear up the question whether this effect is due to a decreased concentration of alkaline phosphatase, or it is the result of a direct inhibition of the enzyme by the hormones. Therefore we have investigated the inhibition of crystalline kidney alkaline phosphatase and liver alkaline phosphatase by estrogenic hormones and some of their derivatives. In these experiments estradiol and estrone showed very slight or no inhibitory effect, while some of their phosphorylated derivatives were very strong inhibitors. These phosphorylated estrogens were not hydrolyzed by the phosphatase.

Experimental. Crystalline alkaline phosphatase from kidneys of juvenile rabbits was prepared by the method of van Thoai *et al* [2]. The final product with a nitrogen content of 13.5 per cent contained a small amount of amorphous material *. In 1 ml hydrolysis mixture, containing 1.2 μg dry enzyme, 0.0079 mmol disodium phenyl phosphate and 0.0008 mmol magnesium sulfate, 26 μg phenol was liberated within 24 minutes at pH 9.67 and 37° C. The enzyme was dissolved in distilled water and kept in a refrigerator. Its activity remained unchanged over a period of 60 days. The liver phosphatase was prepared from livers of juvenile rabbits. No attempts have been made to obtain the enzyme in crystalline form.

Estrone, estradiol and progesterone were dissolved in a solution of cetylpyridi-

* We are indebted to Ing. B. Högberg, AB. Leo, Hälsingborg, Sweden, for the nitrogen determinations.

Table 1. Inhibition of alkaline kidney phosphatase.

Inhibitor	Concentration	Per cent inhibition after min 4	8	16	24
Estrone	$5.7 \cdot 10^{-5}$ *M*	0	0	0	0
Estradiol	$5.7 \cdot 10^{-5}$ *M*	15	0	6	0
Estrone-3-phosphate	$5.0 \cdot 10^{-4}$ *M*	64	77	78	78
—»—	$8.0 \cdot 10^{-5}$ *M*	38	38	41	39
Estradiol-17-phosphate	$8.0 \cdot 10^{-5}$ *M*	8	12	6	3
Estradiol-3,17-diphosphate	$8.0 \cdot 10^{-5}$ *M*	47	47	52	57
Progesterone	$8.0 \cdot 10^{-3}$ *M*	30	30	35	
—»—	$8.0 \cdot 10^{-5}$ *M*	0	6	7	7

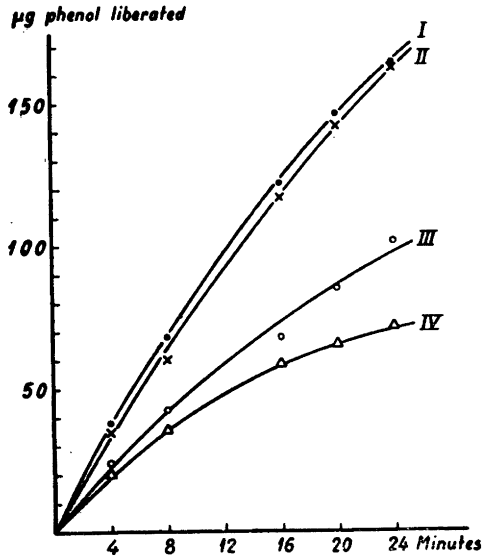

Fig. 1. Inhibition of alkaline kidney phosphatase by different phosphates of the estrogenic hormones.

I: without inhibitor
II: 8 · 10⁻⁵ M estradiol-17-phosphate
III: 8 · 10⁻⁵ M estrone-3-phosphate
IV: 8 · 10⁻⁵ M estradiol-3,17-diphosphate

niumchloride, so that the final reaction mixture contained 80 µg cetylpyridiniumchloride. At this concentration cetylpyridiniumchloride has no inhibitory action on the phosphatase activity.

Estrone-3-phosphate, estradiol-17-phosphate and estradiol-3,17-diphosphate were kindly supplied by Ing. B. Högberg, AB. Leo, Hälsingborg.

For the determination of the phosphatase activity the method of King and Armstrong [3] has been used, in the modification described by Buch and Buch [4]. The amount of phenol liberated was determined after 4, 8, 16 and 24 minutes.

Results. The results of the experiments with the kidney phosphatase are presented in Fig. 1. and Table 1.

Furthermore the inhibitory effect of $1.6 \cdot 10^{-4} M$ estradiol-3,17-diphosphate on alkaline liver phosphatase was investigated. After 4, 8, 16 and 24 minutes the inhibition was 46, 64, 68 and 69 per cent.

From the results presented above it is seen that among the compounds investigated, those phosphorylated in the 3-position have the strongest inhibitory effect on the phosphatase activity. Nothing is known at present about the occurrence of estrogenic hormones as phosphates in living organisms. A discussion of the significance of the present findings together with further experiments will be published later.

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On the Structure of Salt Monohydrates

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The crystal chemistry of salt hydrates is a vast field in Inorganic Chemistry, which has not yet been definitely clarified. Recently, A. Tovborg Jensen [1] has written an excellent, critical review on these substances. He has also given some rules for the neighbourhood of the water molecules in crystals of this type.

Tovborg Jensen states that a water molecule has always negative neighbours