Concerning the Specificity of Alkaline Phosphatases of Human Foetal Bone Tissue

KAUKO K. MÄKINEN and ILKKA K. PAUNIO

Institute of Dentistry, University of Turku, Turku 3, Finland

Provision of orthophosphate for bone formation seems to be a rational function for phosphatases of osteoblasts, although other physiological functions of phosphatases have also been proposed.[1] The problem of specificity of these phosphatases towards phosphorylated compounds is of noticeable practical and theoretical interest. The purpose of this study, of which some reports have been published,[2-4] was to elucidate the substrate specificity of human foetal alkaline phosphatases. The study was made by first partially purifying several phosphatases from human foetal cartilaginous epiphysis, parietal bones, and, for the sake of comparison, from livers. The substrate specificity, or the degree of interaction, was determined by computer calculations.

Table 1. Calculated values of the substrate constant, K_s (in $10^{-5} \times$ M), for the hydrolysis of *p*-nitrophenyl phosphate catalyzed by two alkaline phosphatases purified from human foetal parietal bones (PB). The abbreviations PB II and PB III refer to the enzyme preparations purified as described earlier.[3] The reactions were performed in 0.025 M glycinate buffer, pH 9.2, in the presence of 1 mM $MgCl_2$. The enzyme studied earlier,[3] PB I, also hydrolyzed *p*-nitrophenyl phosphate and α-naphthyl phosphate. The values of K_s for these substrates were $2.80 \pm 0.53 \times 10^{-5}$ M and $1.91 \pm 0.16 \times 10^{-5}$ M, respectively.

Substrate	PB II	PB III
AMP	12.9±1.1	2.3± 0.5
ATP	47.4±9.0	71.4±32.4
p-Nitrophenyl phosphate	3.5±0.7	3.2± 0.5
β-Glyceryl phosphate	10.7±0.8	16.9± 4.1
Glucose 1-phosphate	13.7±1.1	57..0±20.6
α-Naphthyl phosphate	2.0±0.3	18± 0.2
DL-α-Glyceryl phosphate	28.6±1.7	

The materials and methods have been published earlier.[3,5] In this investigation a regression analysis for weighted data was employed for calculating the substrate constant, K_s, by the least squares method described earlier.[5]

Most of the results obtained are summarized in Tables 1 and 2. The following considerations can be made: (1) On the average the degree of interaction between the enzymes and arylphosphates was higher than with other substrates; (2) The values of K_s were essentially the same with the phenyl phosphates regardless of the origin of the enzymes; (3) The degree of interaction, on average, was rather low with adenosine-5′-triphosphate and DL-α-glyceryl phosphate.

In the theory of Michaelis and Menten, the experimentally determined Michaelis constant, K_m (the value of [S] which is found to give half the maximum velocity), equals K_s, the substrate constant, which is the true dissociation constant of the enzyme-substrate complex (*i.e.* of the reaction $E + S \rightleftharpoons ES$). Because the experimentally determined K_m is not necessarily a dissociation constant, and because a clear distinction between the two constants should be made, the substrate constant, K_s, was used in the present study. In earlier reports K_m (determined graphically) was employed.[3] In many cases, however, it is possible to approximate the true dissociation constant to K_m.

The dissociation constant of the enzyme-substrate complex has been used to measure the degree of interaction between the reacting species. In this way the specificity of an enzyme system or single enzyme can be elucidated. The specificity of the sodium-dependent sugar transport system has been investigated by determining graphically the value of K_m.[6] With different phosphates, including various phenyl phosphates, glyceryl phosphates, and ethyl phosphate, values of 0.3–3.0 mM have been obtained for K_s when tested with intestinal phosphatases.[7,8] The alkaline phosphatase of *E. coli* has K_m of 1.1, 1.7, and 0.7 mM, for 5′-AMP, 3′-AMP, and glyceryl 2-phosphate, respectively.[9] Calf intestinal mucosa alkaline phosphatase has values of 11, 0.2, and 17 mM for the three phosphates mentioned, and 0.86 mM for phenyl phosphate.[10,11] In the present study lower values were obtained for phenyl phosphates than reported elsewhere for

Table 2. Calculated values of the substrate constant, K_s, (in $10^{-5} \times M$) for the hydrolysis of some phosphates catalyzed by alkaline phosphatases purified from human foetal tissues. CE = cartilaginous epiphysis; L = liver. I and II refer to the different enzyme preparations purified as earlier described.[3] Reaction conditions were as for Table 1.

Substrate	CE I	CE II	L I	L II
α-Naphthyl phosphate	2.0±0.3	1.4± 0.1	1.4±0.3	1.5±0.3
p-Nitrophenyl phosphate	2.0±0.3	2.2± 0.3	2.2±0.4	2.0±0.7
DL-α-Glyceryl phosphate	10.7±3.2	17.7± 1.1	14.9±3.8	40.2±4.4
β-Glyceryl phosphate	5.7±0.7	5.0± 0.7	10.1±2.0	0.6±0.2
Adenosine 5′-triphosphate	20.5±4.3	42.8±12.8	7.6±3.8	1.4±0.2
Glucose 1-phosphate	14.4±2.2	10.3± 1.1	4.9±0.7	23.7±3.9
Adenosine 5′-monophosphate	8.9±0.7	7.3± 0.9	10.5±1.3	12.4±1.4

other alkaline phosphatases. The values of K_s in the present study can be compared to those determined graphically and given earlier for human foetal alkaline phosphatases.[3,12] The previous and present values were essentially of the same order in most cases. The differences observed can be explained with the aid of the subjectivity of all graphical methods of plotting and by the fact that in graphical methods it is difficult to take substrate inhibition into account.

It appeared that the adenosine phosphates and glyceryl phosphates were poorer substrates for the alkaline phosphatases of the mineralizing tissues studied in the assay conditions used than aryl phosphates. The essentially similar values of K_s obtained for the latter substrates indicate that the catalytic properties of the enzymes involved may be similar in many respects, as also suggested earlier.[2-4]

Support for this investigation was made available to one of the authors (K.K.M.) by Gibbs OY Unilever, Finland. The technical assistance of Mrs. Raili Turta is gratefully acknowledged.

1. Morton, R. K. In Florkin, M. and Stotz, E. H. *Comprehensive Biochemistry*, Elsevier, Amsterdam 1965, Vol. 16, p. 55.
2. Mäkinen, K. K. and Paunio, I. K. *Calc. Tiss. Res. Suppl.* **2** (1968) 53.
3. Mäkinen, K. K. and Paunio, I. K. *FEBS Letters* **3** (1969) 153.
4. Mäkinen, K. K. and Paunio, I. K. *Calc. Tiss. Res. Suppl.* **4** (1970) 40.
5. Mäkinen, K. K., Euranto, E. K. and Kankare, J. J. *Suomen Kemistilehti* **B 42** (1969) 129.
6. Crane, R. K. In Florkin, M. and Stotz, E. H. *Comprehensive Biochemistry*, Elsevier, Amsterdam 1969, Vol. 17, p. 1.
7. Delory, G. E. and King, E. J. *Biochem. J.* **37** (1943) 547.
8. Walker, P. G. and King, E. J. *Biochem. J.* **47** (1950) 93.
9. Heppel, L. A., Harkness, D. R. and Hilmoe, R. J. *J. Biol. Chem.* **237** (1962) 841.
10. Morton, R. K. *Biochem. J.* **65** (1957) 674.
11. Motzok, I. *Biochem. J.* **72** (1959) 169.
12. Mäkinen, K. K. and Knuuttila, M. L. *To be published.*

Received November 18, 1970.