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Alkaline Phosphatase in Various Animal Species and Heat Stability of the Enzyme in Catfish (*Silurus asotus*) Kidney

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ABSTRACT Alkaline phosphatases from the kidneys of three mammals (human, rat and mouse), two birds (chicken and pigeon), one reptile (four-striate snake), one amphibian (frog) and four fish (carp, catfish, rainbow trout and electric ray) were investigated in the following two areas: heat stability of the enzyme and inhibitory effect of amino acids on the enzyme reaction. Only alkaline phosphatase from catfish was heat-stable at 56°C. No significant differences due to phylogenetic relationship were observed among the various animals in these examinations. (*Zool. Mag. 92: 226-230, 1983*)

In a previous study, the activity and substrate specificity of D-amino acid oxidase in the kidney homogenates obtained from various animal species was investigated (Konno and Yasumura, 1981). It was shown that the degree of differences in the substrate specificity of the enzyme of the fish paralleled their phylogenetic relationship (Konno *et al.*, 1982).

Alkaline phosphatase, as well as D-amino acid oxidase, is abundant in kidneys, and the former enzyme has been investigated thoroughly (Fernley, 1971; Fishman, 1974, for a review). It seems, however, that there is no comparative study on alkaline phosphatase among animal species, although the enzymes in different organs have been characterized. In this study, therefore, alkaline phosphatase has been investigated in the kidney homogenates of various animal species, and heat stability of the enzyme and inhibitory effect of amino acids on the enzyme reaction were also examined.

Materials and Methods

Chemicals

Amino acids (A grade), L-histidine $HCl \cdot H_2O$, L-leucine and L-phenylalanine, were purchased from Ajinomoto Co. Ltd. (Tokyo, Japan). L-Homoarginine HCl (guaranteed reagent) was purchased from Nakarai Chemicals Ltd. (Kyoto, Japan). Glycine (reagent grade), disodium phenylphosphate, 4-aminoantipyrine and potassium ferricyanide were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Other chemicals were commercial products of reagent grade.

Animals

The source, size and sex of animals, human (*Homo sapiens*), rat (*Rattus norvegicus*), mice (*Mus musculus*), chicken (*Gallus gallus*), frog (*Rana catesbeiana*), carp (*Cyprinus carpio*), catfish (*Silurus asotus*), rainbow trout (*Salmo gairdneri*) and electric ray (*Narke japonica*), were described in the previous papers (Konno and Yasumura, 1981; Konno *et al.*, 1982). Male four-striate snake (100 cm long, 200 g) (*Elaphe quadrivirgata*) was kindly supplied by Dr. Akiyoshi Mishima, Department of Medical Zoology of this University. Female pigeon (360 g) (*Columba livia domestica*) was obtained from Laboratory of Medical Sciences of this University.

Homogenates

The removed kidneys were minced and then their pieces were homogenized in a

Teflon-glass Potter-Elvehjem type homogenizer in H_2O. A portion of the homogenates was used for the enzyme assay and for the protein assay, according to the method of Lowry *et al.* (1951), with bovine serum albumin as the standard.

Enzyme assay

Alkaline phosphatase activity was measured by the method of Kind-King (1954) with a slight modification by Watanabe *et al.* (1967). A mixture of 50 μl of the homogenates and 1 m*l* of carbonate-bicarbonate buffer (pH 10.15) containing 4.2 mM disodium phenylphosphate and 2.2 mM 4-aminoantipyrine was incubated at 37°C for 10 min, and then the amount of liberated phenol from phenylphosphate was measured spectrophotometrically at 500 nm after the potassium ferricyanide reaction.

Results and Discussion

Table 1 shows the specific activity of alkaline phosphatases in the kidney homogenates of various animal species. The variations in alkaline phosphatase activity due to different individuals were very small, in the

Table 1. Specific activity of alkaline phosphatase in various animal kidneys.

Species	Alkaline phosphatase activity (nmole/min/mg protein) Experiment I	Experiment II
Mammal		
Human	61.8± 0.2	61.2± 0.2
Rat (Wistar)	413 ± 4	422 ± 7
Mouse (DBA)	494 ±16	510 ±10
Mouse (BALB)	564 ±16	580 ±24
Bird		
Pigeon	52.3± 3.2	53.5± 2.8
Chicken	41.7± 3.5	41.0± 1.9
Reptile		
Snake	56.5± 5.6	53.3± 3.2
Amphibian		
Frog	156 ± 4	154 ± 4
Fish		
Carp	160 ± 5	160 ± 5
Catfish	18.2± 0.4	16.1± 0.2
Rainbow trout	28.2± 0.9	28.7± 0.5
Electric ray	109 ± 4	108 ± 3

The protein concentration of the homogenates was in the range of 5—500 μg/50 μl. The value is the mean ± S.D. of three enzyme reactions.

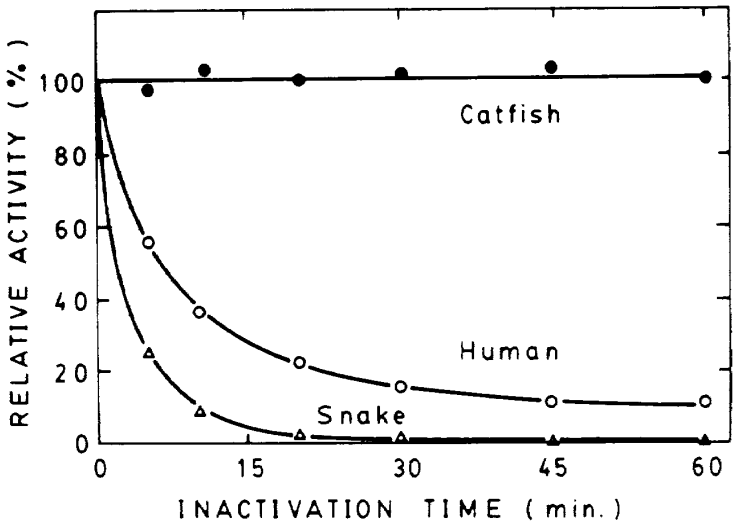

Fig. 1. Heat inactivation curves of alkaline phosphatases in human, four-striate snake and catfish kidneys. The homogenates were preincubated at 56°C for the stated intervals, and the enzyme assay was done at 37°C for 10 min of incubation. The values are given for the experiment, each in triplicate.

range of 10% of the mean value. Large differences were observed among the animals, but it seems that no significant difference due to phylogenetic relationship appeared.

In order to investigate the heat stability of alkaline phosphatase, the homogenates were preincubated at 56°C, and then the enzyme activity was measured at 37°C. The kidney homogenates of human, snake and catfish were preincubated at 56°C for the stated intervals (Fig. 1). Only alkaline phosphatase in the catfish kidney homogenates was heat stable, and the activity was not changed even after 60 min preincubation at 56°C, although, in some experiments, the activity decreased to ~90% of the initial enzyme activity after 60 min preincubation. The enzyme activity of the human and snake kidney homogenates decreased to less than 30% of the initial activity after 15 min preincubation. The enzymes in other animal kidneys were also examined as shown in Fig. 2. Alkaline phosphatases in the chicken, pigeon and frog kidneys were slightly more stable in comparison with the enzymes in the kidneys of human, rat, mice, carp, rainbow trout and electric ray, whose enzyme activity decreased to less than 50% of the initial after 10 min preincubation. It has been thought that only alkaline phosphatases, derived from human hepatomas and placentas, are heat stable (Higashino *et al.*, 1980, for a review). However, we found that alkaline phosphatase in the catfish kidney was also heat stable. In order to explain this difference in the heat stability of the enzyme, further studies will be necessary.

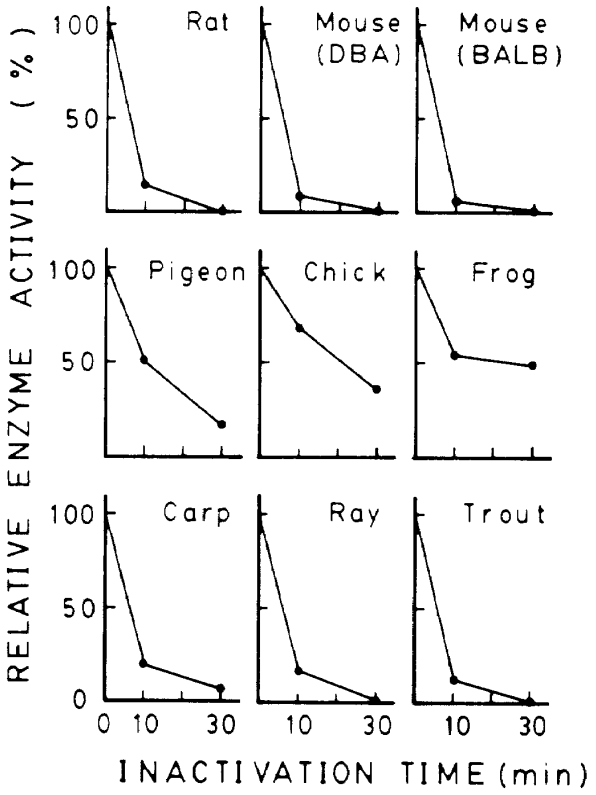

Fig. 2. Heat inactivation curves of alkaline phosphatases in kidneys of various animal species. The homogenates were preincubated at 56°C for 10 or 30 min, and the enzyme assay was done at 37°C for 10 min of incubation. The values are given for the experiment, each in triplicate.

The inhibitory effect of amino acids such as L-homoarginine, L-histidine, L-phenylalanine, L-leucine and glycine, on the enzyme reaction was investigated (Fig. 3). The effect of each amino acid was quite similar among the homogenates obtained from human, snake and catfish kidneys. L-Homoarginine was the most effective, and glycine was the weakest among these amino acids. A similar study was performed in the other animals (Fig. 4). In general, L-homoarginine is the most effective inhibitor of alkaline phosphatase reactions in the liver and bone homogenates, but L-phenylalanine is more effective than L-homoarginine in the placenta and intestine homogenates (Higashino *et al.*, 1980). In addition, it is known that either L-phenylalanine or L-leucine inhibits the reaction of alkaline phosphatase derived from human hepatomas more strongly than L-homoarginine (Higashino *et al.*, 1980).

L-Homoarginine was the strongest inhibitor of alkaline phosphatases obtained

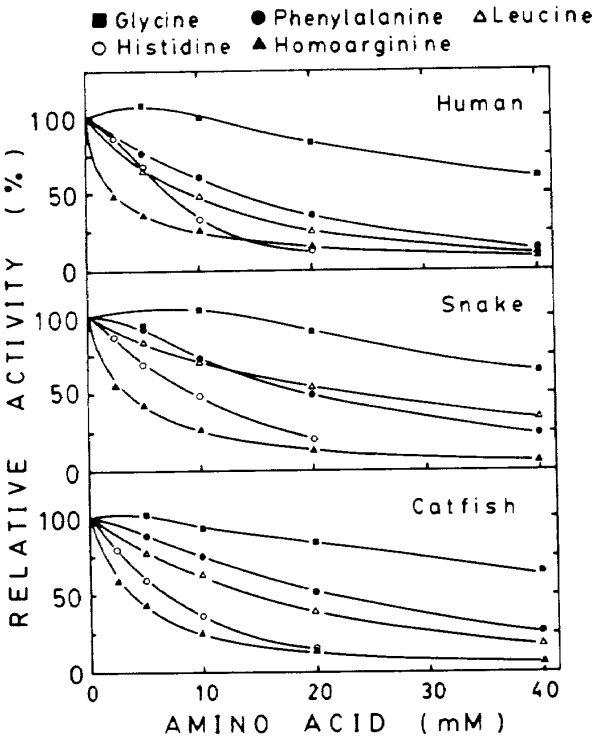

Fig. 3. Inhibitory effect of amino acids at various concentrations on alkaline phosphatase activity in human, four-striate snake and catfish kidneys. The values are given for the experiment, each in triplicate.

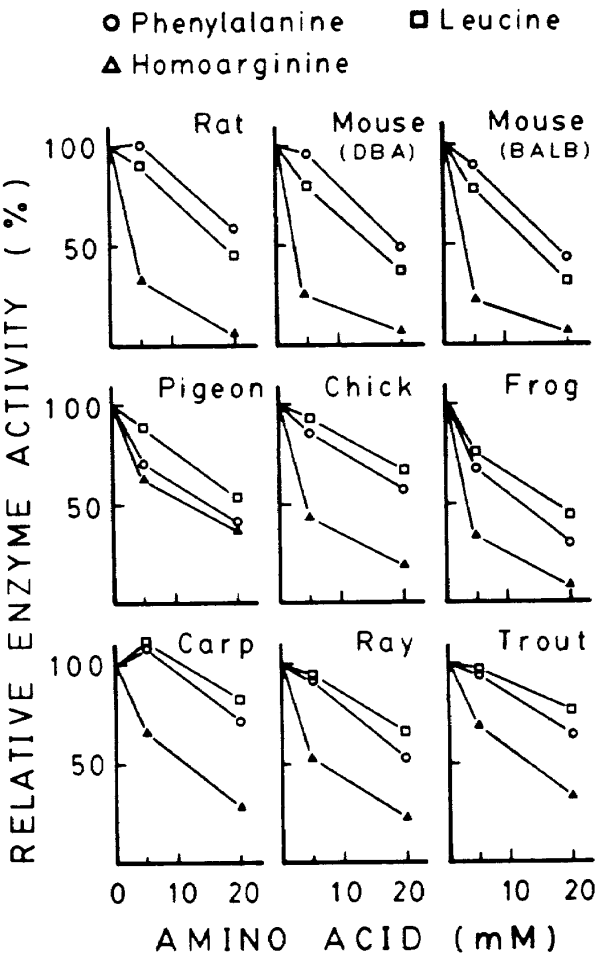

Fig. 4. Inhibitory effect of amino acids at various concentrations on alkaline phosphatase activity in kidneys of various animal species. The values are given for the experiment, each in triplicate.

from the kidneys of various animal species including catfish, among the amino acids examined in this study. No significant relationship was found between the phylogeny and differences in alkaline phosphatases obtained from various animals.

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References

FERNLEY, H. N. (1971) Mammalian alkaline phosphatase. *The Enzymes*, ed. by Boyer, P. D., Academic Press, New York — London, Vol. 4, pp. 417-447.

FISHMAN, W. H. (1974) Perspective on alkaline phosphatase isoenzymes. *Am. J. Med.* **56**: 617-650.

HIGASHINO, K., R. OHTANI AND T. HADA (1980) Cancer and the alkaline phosphatase (in Japanese). *Taisha* **17**: 1519-1531.

KIND, P. N. R. AND E. J. KING (1954) Estimation of plasma phosphatase by determination of hydrolised phenol with amino-antipyrine. *J. Clin. Pathol.* **7**: 322-326.

KONNO, R., S. UCHIYAMA AND Y. YASU-

MURA (1982) Intraspecies and interspecies variations in the substrate specificity of D-amino acid oxidase. *Comp. Biochem. Physiol.* **71B**: 735-738.

KONNO, R. AND Y. YASUMURA (1981) Activity and substrate specificity of D-amino acid oxidase in kidneys of various animals. *Zool. Mag. (Dobutsugaku Zasshi)* **90**: 368-373.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL (1961) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.

WATANABE, K., T. TSUDA AND M. KITAMURA (1967) Estimation of serum alkaline phosphatase. — Modified Kind-King method — (in Japanese). *Rinsho-byori* **15**: 708-712.