海産動物におけるアルカリ性フォスファターゼの組織および種特異性

誌名	日本水産學會誌
ISSN	00215392
巻/号	472
掲載ページ	p. 267-273
発行年月	1981年2月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat

Bulletin of the Japanese Society of Scientific Fisheries 47(2), 267–273 (1981)

Tissue- and Species-Specificity of Alkaline Phosphatase in Marine Animals

Hiroki ABE* and Sachiko GOTOH*

(Received November 7, 1980)

Alkaline phosphatase (EC 3.1.3.1) from various tissues of skipjack, *Katsuwonus pelamis*, and also from hepatic tissues of several marine animals were examined for some enzymatic properties. The total activity of the enzymes from skipjack tissues was high in the pyloric caecum, kidney, and liver, but low in the ordinary and cardiac muscles, and bone. The enzyme activity of fish livers was higher than that of invertebrate hepatopancreas. The optimal pH values of skipjack enzymes significantly differed from the kind of tissues, ranging from 7.3 to 10.2. The hepatic enzymes from most marine animals, however, showed similar optimal values around pH 10, except those from skipjack (pH 8.8) and prawn (pH 8.4). Michaelis constants of the enzymes were found between 0.02 and 0.1 mM against *p*-nitrophenyl phosphate. The inhibitory effects of several effectors, such as *p*-chloromercuribenzoate, L-tryptophan, and L-phenylalanine, on the enzymes depended on the tissues and on the species. The skipjack enzymes seemed to be divided into two groups as to their heat stability. On cellulose acetate electrophoresis, the skipjack enzymes showed different isoenzyme patterns depending on the tissues.

It was concluded from the above data that the tissue-specificity of alkaline phosphatase is much higher than its species-specificity.

During the course of studies on NAD(P)$^+$ hydrolyzing enzymes in skipjack liver,[1-4] we noticed a probable tissue specificity of alkaline phosphatase (EC 3.1.3.1). In addition, the enzymatic properties of the phosphatase from skipjack liver seemed to significantly differ from those of the mammalian counterpart.

The present paper communicates a comparison of properties of skipjack alkaline phosphatases from various tissues in order to clarify the tissue-specificity of the enzyme. The species-specificity of this enzyme among fishes as well as marine invertebrates is also described.

Materials and Methods

Materials

At Tokyo Central Wholesale Fish Market were purchased three to ten live or very fresh specimens of the following five species of fish and three species of marine invertebrate: skipjack, *Katsuwonus pelamis*, common mackerel, *Scomber japonicus*, sardine, *Sardinops melanosticta*, rockfish, *Sevastes inermis*, sweetfish, *Plecoglossus altivelis*, squid, *Ommastrephes sloani pacificus*, scallop, *Patinopecten yessoensis*, and prawn, *Penaeus japonicus*. Livers or hepatopancreas were removed from those specimens of each species, combined, and analyzed as described below. In case of skipjack, other tissues than the liver (refer to Table 1) were also excised and analyzed. Rabbit liver was also used for comparison.

Preparation of Enzyme Solution

Each tissue was homogenized with 3 volumes of 0.15 M KCl and centrifuged at 8,000×g for 20 min at 0°C. The supernatant was brought to 100% saturation with solid ammonium sulfate. The forming precipitate was collected by filtration and dialyzed overnight against 0.05 M Tris-HCl buffer, pH 7.8. After centrifugation, the supernatant obtained was used as enzyme solution. A partially purified enzyme preparation from skipjack[1,4] was also employed for electrofocusing analysis.

Enzyme Assay

Alkaline phosphatase activity was determined essentially by the method reported previously.[4] An incubation mixture contained 0.07 mM *p*-nitrophenyl phosphate, 0.15 M Tris-HCl buffer, pH 8.0–9.8, or cyclohexylaminopropane sulfonic acid-HCl buffer, pH 9.8–11.0, 10 mM $MgCl_2$, 0.01 to

* Lab. of Chem., Fac. of Home Econom., Kyoritsu Women's Univ., Motohachioji, Hachioji 193, Tokyo (阿部宏喜・後藤砂智子：共立女子大学家政学部).

0.1 m*l* of enzyme solution in a final volume of 3.0 m*l*. Liberated *p*-nitrophenol was followed spectrophotometrically at 400 nm. The enzyme activity was expressed as μmol *p*-nitrophenol/h·g tissue.

Measurement of Thermal Inactivation

Aliquots of each enzyme solution prepared as above were heated at 40, 50, and 60°C for 15 min and the remaining activities were assayed.

Electrophoresis

Cellulose acetate electrophoresis was carried out for 30 min using Cellogel (Chemetron) in 0.069 M veronal buffer, pH 8.6 at 1 mA/cm. Alkaline phosphatase activity was stained by incubating the Cellogel strip in 5 m*l* of 0.2 M Tris-HCl buffer, pH 9.0 containing 2 mg naphthol AS-MX phosphate (Sigma), 5 mg Fast Violet B salt and 5 mg $MgCl_2$.

Analytical electrofocusing in polyacrylamide gel was performed using LKB Multiphor 2117 system. Ampholine PAG plate (pH 3.5–9.5) was employed as a gel slab. As the cathodic and anodic electrode solutions, 1 M NaOH and 1 M H_3PO_4 were used respectively. The electrofocusing was carried out for about 1.5 h at 0°C at a constant voltage of 1,000 V. After electrophoresis, the gel was stained for alkaline phosphatase as described above.

Results and Discussion

Total Activity

Alkaline phosphatase activity levels in various tissues of skipjack as well as in hepatic tissues of other fishes and invertebrates are shown in Table 1. The activity of skipjack enzyme was high in the pyloric caecum, kidney, and liver, but low in the ordinary and cardiac muscles, and bone. The dark muscle showed a much higher activity than other muscles. For mammals, the activity has been reported to be high in intestinal mucosa, placenta, kidney, and bone, and low in liver, lung,

Table 1. Total activities, optimal pH's and Michaelis constants of alkaline phosphatases from skipjack tissues and from hepatic tissues of several marine animals

Sources	Optimal pH	Total activity (μmol/h·g tissue)	K_m* (mM)
Skipjack			
Pyloric caecum	9.8–10.0	522	0.061
Kidney	9.5	501	0.045
Liver	8.8	288	0.0081
Dark muscle	9.2	125	0.034
Intestine	9.3	40	0.018
Ovary	9.8–10.0	30	0.055
Testis	9.8–10.0	26	0.078
Serum	10.2	13	0.048 (pH 9.4), 0.035 (pH 10.2)
Ordinary muscle	9.6	5.3	0.263
Cardiac muscle	7.3	2.7	0.403 (pH 7.3), 0.033 (pH 9.4), 0.044 (pH 10.0)
Bone	9.4	1.4	0.031
Common mackerel			
Liver	9.8–10.0	21	0.077
Sardine			
Liver	10.0	208	0.060
Rockfish			
Liver	10.0	56	0.102
Sweetfish			
Liver	10.0	49	0.064
Squid			
Hepatopancreas	10.2	4.1	—
Scallop			
Hepatopancreas	9.8	0.6	—
Prawn			
Hapatopancreas	8.4	4.4	0.111
Rabbit			
Liver	10.4	3.2	0.068

* Michaelis constant at optimal pH of each enzyme.

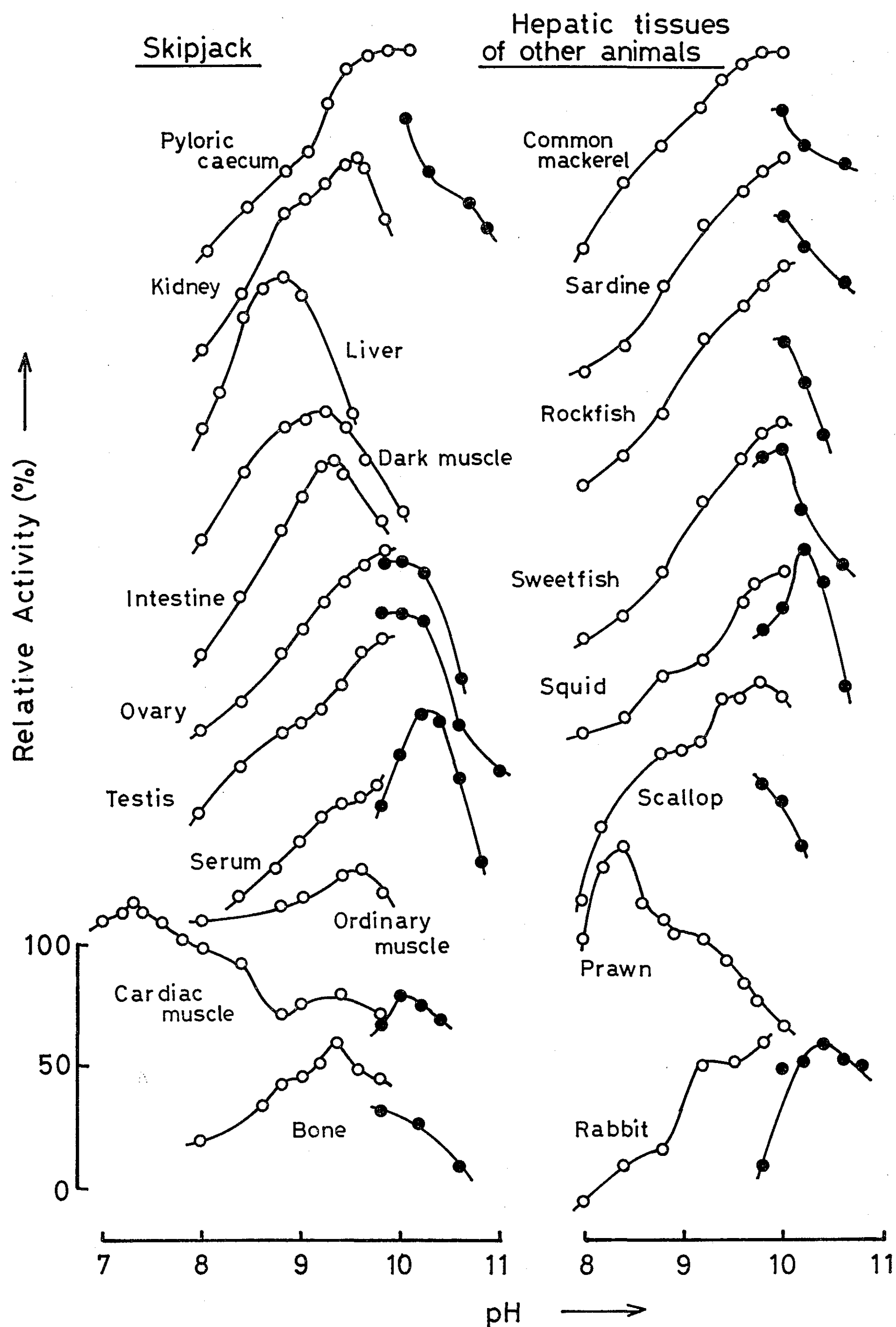

Fig. 1. pH-activity curves of alkaline phosphatases from various tissues of skipjack and from hepatic tissues of several marine animals.
○—○; Activity in 0.15 M Tris-HCl.
●—●; Activity in 0.15 M cyclohexylaminopropane sulfonic acid-HCl.

muscle, and spleen.[5] The activity in skipjack pyloric caecum is almost identical to that in humun placenta (612 μmol/h·g tissue).[6] It is noteworthy that in the skipjack the activity is rather high in the liver and low in the intestine and bone compared with the case of mammals. As a whole, the activity of alkaline phosphatase of fish liver was higher than that of invertebrate hepato-

pancreas which exhibits the activity comparable to that of rabbit liver.

Optimal pH

As shown in Fig. 1, pH-activity curves of alkaline phosphatases from these sources significantly differed from each other, showing the optima between pH 7.3 to 10.2 (Table 1). In case of skipjack, the enzymes from several tissues showed a slight shoulder at around pH 9. The pH dependence of cardiac muscle enzyme was complicated and specific, suggesting the presence of multiple enzymes. Skipjack liver enzyme showed a sharp and typical curve having an optimum at pH 8.8. Skipjack alkaline phosphatases thus showed significantly high tissue-specificity as to optimal pH.

The hepatic enzymes from various fishes and marine invertebrates, however, showed a rather similar profiles as to pH-activity curve. The optimal pH's were at around 10 except for skipjack and prawn enzyme of which the optima appeared at pH 8.8 and 8.4, respectively. Some of them showed a shoulder at pH 9.0–9.5. The enzyme from rabbit liver resembled to that from marine animals in respect of the pH-activity curve though the optimum was a little higher (pH 10.4). Therefore, the tissue-specificity of this enzyme seems to be

Table 2. Effect of several effectors on alkaline phosphatase activity of marine animal tissues

Sources	Relative activity (%)									
	None	L-Cysteine (0.67 mM)	2-Mercaptoethanol (0.043 mM)	PCMB*1 (0.67 mM)	Urea (2.67 mM)	EDTA*2 (3.33 mM)	$ZnCl_2$ (1.33 mM)	Arsenate (0.67 mM)	L-Tryptophan (1.33 mM)	L-Phenylalanine (4.00 mM)
Skipjack										
Pyloric caecum	100	0	0	100	62	43	13	0	98	98
Kidney	100	6	4	60	31	80	23	0	90	80
Liver	100	10	3	12	12	12	87	0	100	100
Dark muscle	100	18	17	80	20	66	51	0	79	83
Intestine	100	19	0	74	21	79	44	0	100	95
Ovary	100	10	13	100	44	60	42	8	72	72
Testis	100	0	0	100	63	57	13	0	78	66
Serum	100	0	0	148	54	43	39	0	76	64
Ordinary muscle	100	100	100	—	0	90	8	0	100	100
Cardiac muscle	100	50	92	100	72	—	—	0	83	81
Bone	100	47	84	123	35	52	67	11	100	100
Common mackerel										
Liver	100	27	0	62	90	65	25	6	163	81
Sardine										
Liver	100	1	1	93	89	53	10	5	76	68
Rockfish										
Liver	100	27	7	127	92	66	66	6	80	70
Sweetfish										
Liver	100	41	3	88	102	53	53	10	79	42
Squid										
Hepatopancreas	100	48	9	26	87	65	—	26	122	135
Scallop										
Hepatopancreas	100	0	0	50	100	50	—	0	200	475
Prawn										
Hepatopancreas	100	43	57	57	86	43	—	29	100	171
Rabbit										
Liver	100	10	11	90	69	79	25	0	66	51

*1 *p*-Chloromercuribenzoate, *2 Ethylenediaminetetraacetate.

clearer than the species-specificity, as far as the present data are concerned. These rather complicated pH-activity profiles indicate again the occurrence of isoenzymes, as further discussed below.

Michaelis Constants

Michaelis constants (K_m) of these alkaline phosphatases against *p*-nitrophenyl phosphate are shown in Table 1. The constants were quite similar within a range from 0.02 to 0.1 mM regardless of enzyme sources, except for that of skipjack liver (0.0081 mM which is almost identical to that reported previously[4]) and of ordinary muscle (0.263 mM). These values are somewhat lower than those of human enzymes (0.1–1 mM).[7]

Effect of Various Inhibitors

As reported previously,[4] various compounds affected the activity of skipjack liver alkaline phosphatase. The influence on those phosphatases of various effectors including several inhibitors which strongly affected skipjack liver enzyme was examined. As shown in Table 2, two reducing agents, L-cysteine and 2-mercaptoethanol, markedly inhibited the enzymes from most sources. Exceptionally, the enzymes from skipjack ordinary and cardiac muscles and bone, and from prawn hepatopancreas were not or hardly affected. *p*-Chloromercuribenzoate slightly inhibited the enzymes from kidney, dark muscle, intestine of skipjack and from the hepatic tissues of common mackerel, sardine, sweetfish, and the invertebrates. This compound, however, showed no effect on the enzymes from pyloric caecum, ovary, testis, and cardiac muscle, whereas it rather activated those from skipjack serum and bone, and rockfish liver. Urea also strongly inhibited the enzymes from skipjack kidney, liver, dark muscle, intestine, ordinary muscle, and bone. On the other hand, little or no inhibition by urea was observed on the enzymes from common mackerel, sardine, rockfish, sweetfish, and the invertebrates. Ethylenediaminetetraacetate inhibited effectively or slightly the alkaline phosphatases from all these sources. Zinc chloride[8,9] and arsenate,[10] known to be specific inhibitors of the enzymes from skipjack liver[4] and mammals, inhibited the enzymes from these marine animals strongly without any exception. L-Tryptophan[11] and L-phenylalanine,[12] known as species- and stereo-specific inhibitors of alkaline phosphatase in mammals, exerted no effect on the enzymes from skipjack pyloric caecum, liver, intestine, ordinary muscle, and bone. The hepatopancreatic enzymes from the invertebrates were activated by both amino acids. The degree of activation was as high as 475% in case of the scallop enzyme. The common mackerel liver enzyme was also activated by L-tryptophan significantly.

These data, especially the data with *p*-chloromercuribenzoate and amino acids, clearly show the presence of species- and tissue-specificity of the

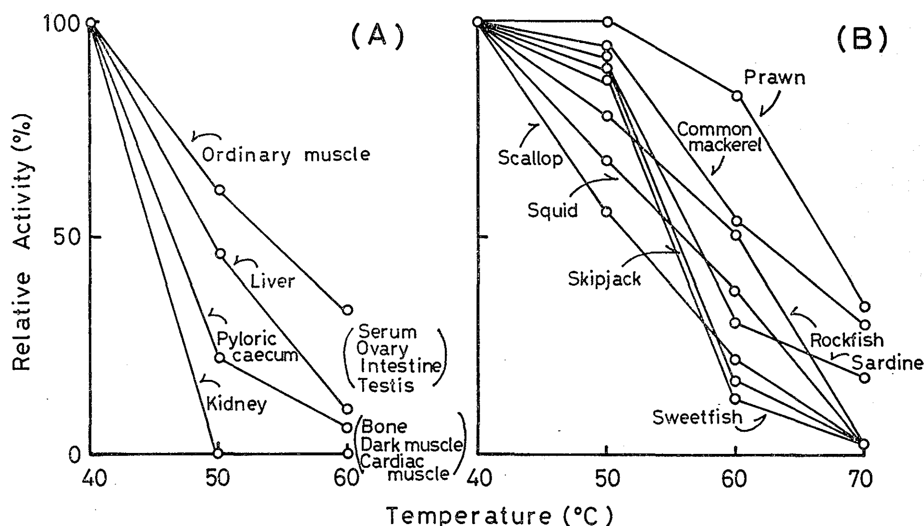

Fig. 2. Thermal inactivation of alkaline phosphatases from various tissues of skipjack and from hepatic tissues of several marine animals.
(A); Skipjack tissues. Heating time: 15 min.
(B): Hepatic tissues of marine animals. Heating time: 1 min.

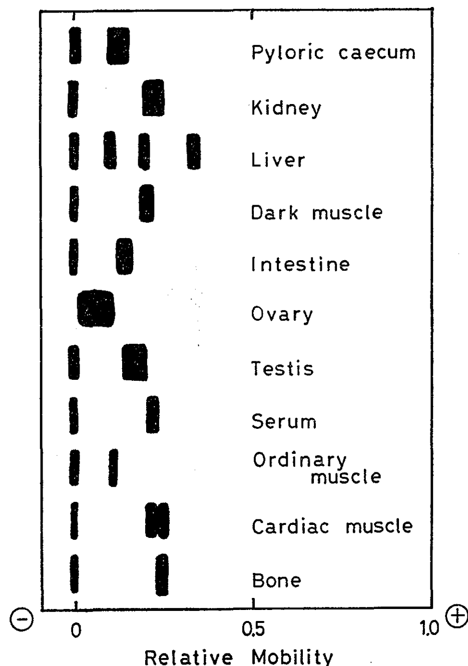

Fig. 3. Schematic cellulose acetate electropherograms of alkaline phosphatases from skipjack tissues.

Refer to the text for the conditions applied.

marine alkaline phosphatase. Skipjack liver enzyme was specific in behaviors to these inhibitors.

Thermal Inactivation

As shown in Fig. 2 (A), skipjack alkaline phosphatases examined here may be classified into two groups in respect of thermostability: One group consisting of heat stable enzymes whose remaining activities were 50 to 60% on heating at 50°C, and another one of heat labile enzymes. The enzymes from ordinary muscle, serum, ovary, testis, intestine, and liver belong to the former group, and those from pyloric caecum, bone, dark muscle, kidney, and cardiac muscle belong to the latter.

With the hepatic enzymes from various piscine and invertebrate sources, the period of heating was reduced to 1 min, because of rather quick inactivation of several enzymes. Their temperature-activity curves roughly resembled each other, prawn enzyme being the most stable (Fig. 2 (B)).

Occurrence of Isoenzymes

Cellulose acetate electropherograms of skipjack alkaline phosphatases are schematically shown in Fig. 3. With all the enzymes except that of ovary, one band remained at the origin and another broad band migrated toward the anode. Exceptionally, 4 bands appeared in case of liver enzyme, and a very broad band in case of ovary enzyme.

Electrofocusing was carried out on some of those enzymes including the partially purified preparation from skipjack liver.[1,4] As shown in Fig. 4, a broad band appeared with most samples. However, the partially purified skipjack liver phosphatase showed many isoenzyme bands. The alkaline phosphatases of marine animals are considered by analogy to consist of many isoenzymes.

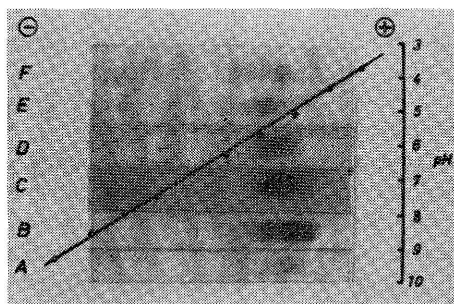

Fig. 4. Isoelectrofocusing of alkaline phosphatases from livers of several fishes.

A; Crude enzyme of skipjack, B; Partially purified skipjack enzyme (about 30-fold purification[1]), C,D,E,F: Crude enzymes from common mackerel, sardine, rockfish, and sweetfish, respectively.

Refer to the text for the conditions applied.

The alkaline phosphatases of marine animals showed different enzymatic properties depending on the kind of tissues and species. The tissue-specificity was higher than the species-specificity in several properties. Either specificity, however, may be due to the isoenzyme pattern that is specific to the tissues and species. Although the function of alkaline phosphatase has not been elucidated fully, the species- and tissue-specificity may suggest that the enzyme play a multiple role in these tissues and species.

The authors are indebted to Professor K. HASHIMOTO, Fac. of Agr., The Univ. of Tokyo, for reading the manuscript. This study was partly supported by a grant from the Ministry of Education, Science and Culture.

References

1) H. ABE, C. SHIMIZU, and F. MATSUURA: *Bull. Japan. Soc. Sci. Fish.*, **42**, 713–720 (1976).
2) H. ABE, C. SHIMIZU, and F. MATSUURA: *Bull.*

Japan. Soc. Sci. Fish., **43**, 469–476 (1977).
3) H. ABE, C. SHIMIZU, and F. MATSUURA: *Bull. Japan. Soc. Sci. Fish.*, **44**, 279–284 (1978).
4) H. ABE, C. SHIMIZU, and F. MATSUURA: *Bull. Japan. Soc. Sci. Fish.*, **44**, 285–292 (1978).
5) H. N. FERNLEY: in "The Enzymes" (ed. by P. D. BOYER), Vol. 4, Academic Press, New York, 1971, pp. 417–447.
6) D. R. HARKNESS: *Arch. Biochem. Biophys.*, **126**, 503–512 (1968).
7) T. KOMODA and Y. SAKAGISHI: *Protein, Nucleic Acid and Enzyme*, **24**, 131–144 (1979).
8) D. R. HARKNESS: *Arch. Biochem. Biophys.* **126**, 513–523 (1968).
9) J. G. GEORGATSOS: *Arch. Biochem. Biophys.*, **121**, 619–624 (1967).
10) R. H. EATON and D. W. MOSS: *Biochem. J.*, **102**, 917–921 (1967).
11) C. W. LIN, H. G. SIE, and W. H. FISHMAN: *Biochem. J.*, **124**, 509–516 (1971).
12) N. K. GHOSH and W. H. FISHMAN: *J. Biol. Chem.*, **241**, 2516–2522 (1966).