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ALKALINE PHOSPHTASES FROM INTESTINES OF FISHES OF TAIWAN*

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Jei-Fu Shaw and Hua-Hsien Chu (1985) Alkaline Phosphatases from Intestines of Fishes of Taiwan. *Bull. Inst. Zool., Academia Sinica* **24**(2): 297-302. Several fish intestines were rich sources of alkaline phosphatase. Five fish intestines studied in this report were: ribbon fish, sharptoothed eel, Alaska pollack, Tilapia and milk fish. The alkaline phosphatase activities per gram acetone powder were 30.4, 10.0, 25.6, 18.6 and 27.0 units respectively. The specific activities of crude enzyme were 1.96, 0.32, 1.09, 1.45 and 1.67 units per mg protein respectively. The optimal pH were 11, 10.0, 10.5, 10.5 and 10.5 respectively. The optimal temperature were 55, 60, 35, 55 and 55°C respectively. The inactivation rate constants measured at 37°C in one hour period were: 0, 0.0058, 0.01, 0.013 and 0.0007 min^{-1} respectively.

Alkaline phosphatases (E. C. 3. 1. 3. 1) are widely distributed in nature. It has been found in bacteria, fungi, Indian leech, develping Drosophila, surlf clam, mammals and fishes (Reid and wilson, 1971; Fernley, 1971; Bodansky *et al.*, 1931). Isozymes are distributed in liver, bone, placenta, kidney and small intestine etc. and they are possibly related to the different physiological function (Bodansky *et al.*, 1931). The order of activities from different tissues are intestinal mucosa-placenta > kidney - bone > liver - lung-spleen (Fernley, 1971). The alkaline phosphatase may be involved in the transport of several substances such as long-chain fatty acid, choline and calcium in the intestinal mucosa (Norman *et al.*, 1970; Pekarthy *et al.*, 1972; Koyama *et al.*, 1983).

Alkaline phosphatase is also a commercially important enzyme. It is used quite extensively in genetic engineering and enzyme immunotechnique (Maniatis *et al.*, 1982; Mayer and Walkgr, 1980; Avrameas, 1976). In genetic engineering work, terminal 5′-phosphate of DNA or RNA can be removed by alkaline phosphatase and this is useful for DNA sequencing or preventing self-ligation in DNA recombination. In enzyme immunotechnique, alkaline phosphatase is the commonly used enzyme for coupling to antibodies. The enzyme-labelled antibodies can be used in localization of cellular constituents and quantitation of antigens.

In this report, we studied some properties of alkaline phosphatase from some fish intestines (fish processing wastes) produced in Taiwan and evaluted the potential for commercial use.

MATERIAL AND METHODS

Materials

Fish intestines taken from ribbon fish (*Trichiurus lepturus*), sharptoothed eel (*Muraensox cinereus*), Alaska pollak (*Theragra*

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chalcogramma) were obtained from Ta-Yang, Ta Jung and Hai-Li Marine Food Processing Co. respectively. Intesetines of Tilapia (*Oreochromis mossambica*) and Milk fish (*Chanos chanos*) were obtained from local market. Zinc chloride, magnesium chloride, glycine, hexamethylene diamine were obtained from Merk Co. *p*-Nitrophenyl phosphate and Tris-HCl were obtained from Sigma Co. Protein assay reagents was purchased from Bio-Rad Co., other chemicals were of reagent grade.

Extraction of fish intestinal alkaline phosphatase

All procedures were carried out at 4°C except where indicated. The fish intestine was minced and then homogenized in 10 mM Tris-HCl buffer (pH 8.0) for 5 minutes by Polytron homogenizer. Precooled acetone (−20°C) was then added slowly into the homogenate with stirring. The suspension was then filtered with Whatman No. 1 paper. The procedure was repeated twice. This acetone powder was stored in freezer until use.

Acetone powder was added to 2 volume of 10 mM Tris-HCl (pH 8.0) and 1 volume of 100% butanol and stirred for 2 hours, then centrifuged at refrigerated centrifuge (10,000 g ×30 min). The lower fraction of supernatant was the enzyme extract.

Enzyme assay

Alkaline phosphatase activity was measured at 37°C with *p*-nitrophenyl phosphate (6 mM) in a buffer containing 0.1 M glycine (pH 10.4), 1.0 mM $ZnCl_2$ and 1.0 mM $MgCl_2$. Hydrolysis was followed with Perkin-Elmer λ-5 spectrophotometer at 410 nm. The molar extinction coefficient used was 16,300 $M^{-1}. cm^{-1}$. One unit of activity is defined as that amout of enzyme which hydrolyzes 1 μ mole of substrate per min.

Protein petermination

Protein concentration was determined according to Bradford (11) with bovine plasma albumin as standard.

Stabilities of enzyme

Enzyme in a buffer containing 0.1 M glycine (pH 10.4), 1.0 mM $ZnCl_2$ and 1.0 mM $MgCl_2$ was preincubated at 37°C for various time intervals, the *p*-nitrophenyl phosphate (6 mM) was added to assay the enzyme activity.

RESULTS AND DISCUSSION

The alkaline phosphatase activities were different in various fish intestines. As shown in Table 1, the enzyme activity per gram acetone powder of intestine varied from 10 to 30 units. The specific activity of crude enzyme extract varied from 0.32 to 1.96 units/mg. Ribbon fish appeared to contain highest intestinal alkaline phosphatase activity.

Fig. 1 showed the pH-activity profile of various fish intestinal enzyme. The optimal pH for the enzymes from different fishes were: ribbon fish, 11; sharp-toothed eel, 10; Alaska pollack, 10.5; Tilapia, 10.5; milk fish, 10.5. pH changes greatly affect the enzyme activity. One pH unit deviation from optimal pH can reduce over 50% of enzyme activity.

Fig. 2 showed the temperature-activity profile of various fish enzyme. The optimal temperatures were; ribbon fish, Tilapia and milk fish, 55°C; sharp-toothed eel, 60°C; Alaska pollack, 35°C. Since the optimal

TABLE 1

Alkaline phosphatase activities of fish intestines and the specific activity of crude enzyme extract

Alkaline phosphatases from acetone powder of various fish intestines were extracted with two volumes of 10 mM Tris (pH 8.0) and one volumes of butanol as described in "Methods". The enzyme activities in the aqueous phase supernatant were assayed at 37°C and pH 10.4 as described in "Methods".

Source	Activity (units/g of acetone powder)	Specific Activity (units/mg)
Ribbon fish	30.4	1.96
Sharp-toothed eel	10.0	0.32
Alaska pollack	25.6	1.09
Tilapia	18.6	1.45
Milk fish	27.0	1.67

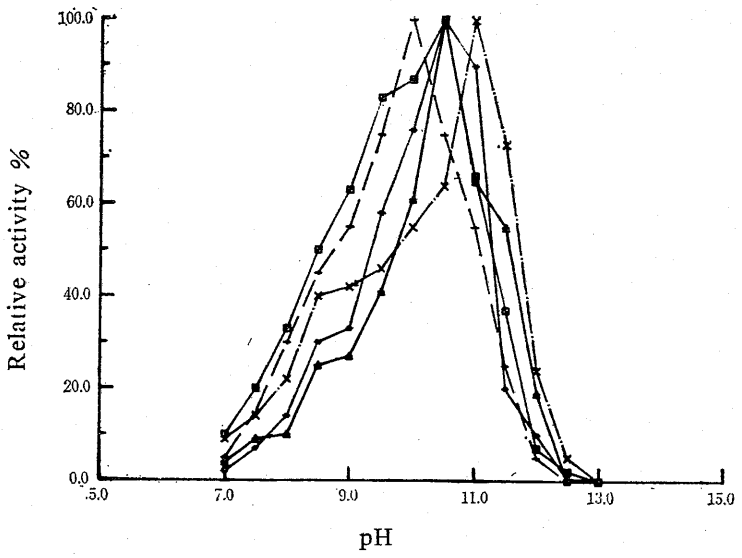

Fig. 1. pH-activity profile of fish intestinal alkaline phosphatases. The buffer used in the enzyme assay are Tris and Glycine for pH 7 to 11, hexamethylenediamine for pH 11.5 to 13.Alaska pollack (*); Tilapia (△); sharp-toothed eel (+); milk fish (□); ribbon fish (×). The enzyme activities at 100% relative activities for ribbon fish, sharp-toothed eel, Alaska pollack, Tilapia and milk fish are 3.08, 0.43, 1.09, 1.45, 1.67 units/mg respectively.

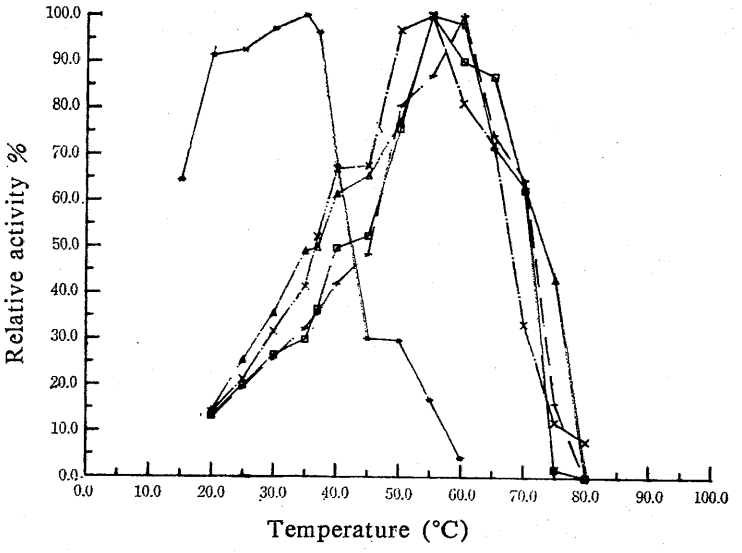

Fig. 2. Teemperature-activity profile of fish intestinal alkaline phosphatases. The symbols denoting five different fish intestines are the same as described under Fig. 1. Tee enzyme activities at 100% relative activities at 100% relative activities for ribbon fish, sharp-toothed eel, Alaska pollak, Tilapia and milk fish are 3.76, 0.9, 1.13, 2.91, 4.59 units/mg respectively.

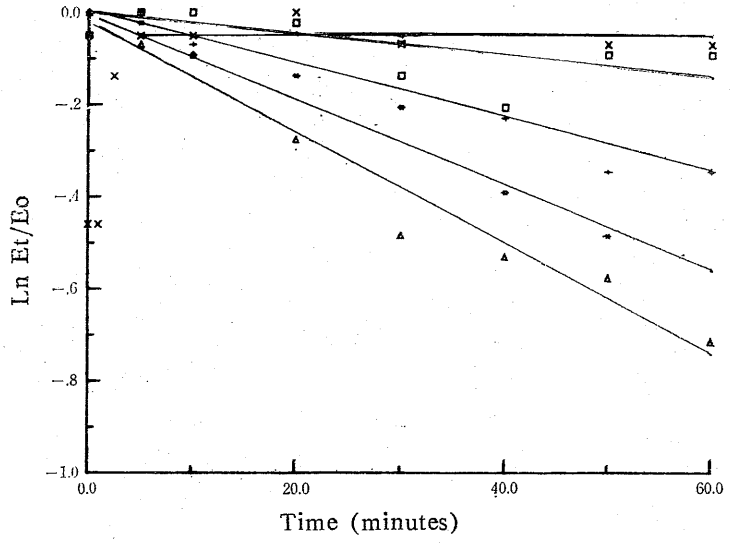

Fig. 3. Stabilities of fish intestinal alkaline phosphatases. The experiment was carried out at 37°C and pH 10.4 as described in Methods. The symbols denoting different fish intestines are the same as described under Fig. 1.

temperature was measured in short time period (2 min.), it must be understood that for longer incubation time, the values of optimal temperature might be lower as a result of enzyme inactivation. However, the optimal temperature of the enzymes generally correlated with the living environment of the fishes. Milk fish, Tilapia, sharptoothed eel and ribbon fish live in warm water (Tien, 1977; Hong, 1979), the optimal temperatures of their intestinal alkaline phosphatase were similar (around 55°C). Alaska pollack generally lives in the northern area of Pacific and Atlantic ocean with water temperature 0°–16°C and therefore the optimal temperature of its enzyme was much lower (35°C).

Fig. 3 showed the stabilities of alkaline phosphatase at 37°C and pH 10.4. The inactivation generally followed first order kinetics. The estimated inactivation rate constants were: sharp-toothed eel, 0.0058 min^{-1}, milk fish. 0.0001 min^{-1}, Alaska pollack, 0.01 min^{-1}; Tilapia, 0.013 min^{-1}. The enzyme from ribbon fish showed no significant enzyme inactivation at this condition. The inactivation of crude enzyme might be either due to heat inactivation or protease inactivation or both. It is interesting to see that only the alkaline phosphatase from ribbon fish needed 10 min incubation at 37°C to reach maximal activity. Further studies on purified enzyme are needed to explain these observations.

Judging from the enzyme activity (Table 1) and stabilities (Fig. 3), it is clear that intestinal alkaline phosphatase of ribbon fish and milk fish are most suitable for commercial use. The annual catch of ribbon fish and annual production of milk fish from aquaculture in Taiwan are estimated to be twenty four and thirty thousand tons respectively (Chen, 1984; Chen 1984). The wasted fish intestines appeared to be very good resources for producing high value enzymes such as alkaline phosphatases. Purifications of alkaline phosphatase and other useful enzyme from fish intestines are currently under investigation.

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臺灣產數種魚腸中之鹼性去磷酸酶

蕭 介 夫　邱 華 賢

數種魚腸中含有豐富之鹼性去磷酸酶。本報告研究之臺灣生產五種魚腸白帶魚、海鰻、明太鱈、吳郭魚、虱目魚。其丙酮粉末之酵素活性爲每克含 30.4, 10.0, 25.6, 18.6 及 27.0 單位。其各自之粗酵素比活性爲 1.96, 0.32, 1.09, 1.45 及 1.67 單位/毫克蛋白質。其各自之最適反應 *p*H 值爲 11, 10.0, 10.5, 10.5 及 10.5。其各自之最適反應溫度爲 55, 60, 35, 55 及 55°C。其在 37°C 下一小時內測定之酵素失活速率常數爲 0, 0.0058, 0.01, 0.013 及 0.0007 min^{-1}。