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Comparison of Properties of Human Intestinal and Placental Alkaline Phosphatase[1]

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Enzymic properties of purified alkaline phosphatases (*ortho*-phosphoric monoester phosphohydrolase, E.C. 3.1.3.1) from human small intestinal mucosa and human placenta were compared. Intestinal alkaline phosphatase had similar properties to those of placental alkaline phosphatase with respect to inhibition by L-phenylalanine, isoelectric point, pH stability, optimum pH, and Michaelis constant. However, difference in enzymic properties was found with respect to the molecular weight, heat stability, and the degree of inhibition by phosphate ion.

An alkaline phosphatase (E.C. 3.1.3.1) is present in the serum and its activity can be separated into six active bands by electrophoresis on agar gel plate.[3] At present, serum alkaline phosphatase is thought to be originated from the liver, bone, small intestine, and placenta.[4] It is very important as the cause of various diseases.[5] However, enzymic properties of alkaline phosphatase from various organs have been veiled and unable to compare with each other, since the purified enzymes were not available.

In the previous paper, we reported the purification alkaline phosphatase from human placenta[6] and from human intestine.[7] In the present work, alkaline phosphatase from human ntestine with that from placenta was compared to study the detailed enzymic properties of each enzyme.

Material and Method

Assay of Alkaline Phosphatase——*p*-Nitrophenyl phosphate was used as a substrate.[8] A mixture of 1 ml of 10mM substrate solution and 3 ml of glycine–KCl–KOH buffer (pH 10.5) was preincubated at 37°. One ml of the enzyme solution was added and the enzyme reaction was stopped by adding 2 ml of 0.1 N NaOH. The absorbancy was determined at 430 nm.

Isoelectric Focusing——Isoelectric focusing was carried out as described by Vesterberg and Svensson,[9] using 1% carrier ampholyte (pH 3—10) at the constant voltage of 800 V for 48 hr.

Human Placental Alkaline Phosphatase——Crude enzyme was obtained from the fresh human placenta by the modification of the method of Morton.[10] This was purified by ammonium sulfate fractionation and passed through a columns of diethylaminoethyl (DEAE)-cellulose, Sephadex G-150, and carboxymethyl (CM)-cellulose, as already described.[6] The disc electrophoresis revealed a single band stainable with Amido Black 10B on polyacrylamide gel.

1) This work was reported at the Symposium on Chemical Physiology and Pathology, Osaka, December, 1972; this paper forms part CIV of "Studies on Enzymes". Part CIII: M. Sugiura, M. Isobe, K. Hirano, S. Iino, H. Suzuki, and T. Oda, *Chem. Pharm. Bull.* (Tokyo), **23**, 1537 (1975).
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3) H. Suzuki, M. Yamanaka, and T. Oda, *Ann. N. Y. Acad. Sci.*, **166**, 811 (1969).
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6) S. Iino, K. Abe, T. Oda, H. Suzuki, and M. Sugiura, *Clin. Chim. Acta*, **42**, 161 (1972).
7) M. Sugiura, M. Isobe, K. Hirano, S. Iino, H. Suzuki, and T. Oda, *Chem. Pharm. Bull.* (Tokyo), **23**, 1537 (1975).
8) O.A. Bessey, O.H. Lowry, and M.J. Brock, *J. Biol. Chem.*, **164**, 321 (1946).
9) O. Vesterberg and H. Svensson, *Acta Chem. Scand.*, **20**, 820 (1966).
10) R.K. Morton, *Biochem. J.*, **34**, 1246 (1954).

Human Small Intestinal Alkaline Phosphatase——Alkaline phosphatase was extracted from human small intestine by the modified Morton's butanol method[10] and was purified by ammonium sulfate fractionation and by passing through the columns of DEAE-cellulose, CM-cellulose, and Sephadex G-200, as previously described.[7] The purified enzyme exhibited a single protein band on polyacrylamide gel in disc electrophoresis.

Results

Isoelectric Point

The isoelectric point was determined by isoelectric focusing using a carrier ampholyte (pH 3—10). As shown in Fig. 1, the isoelectric point of intestinal alkaline phosphatase was

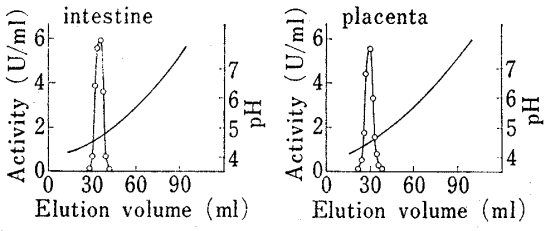

Fig. 1. Isoelectric Focusing Pattern of Alkaline Phosphatase from Human Small Intestinal Mucosa and Human Placenta

column volume: 110 ml, —○— alkaline phosphatase activity —— pH

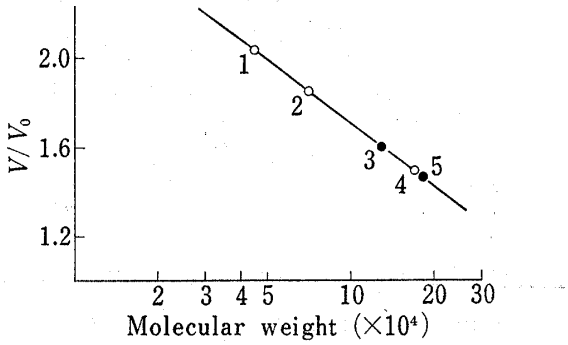

Fig. 2. Determination of the Molecular Weight of Alkaline Phosphatase by Gel Filtration on Sephadex G-200

1: ovalbumin, 2: bovine serum albumin, 3: placental alkaline phosphatase, 4: γ-globulin, 5: intestinal alkaline phosphatase, column size: 2.8×65 cm, buffer: 10 mM Tris-HCl (pH 7.5)

similar to that of placental alkaline phosphatase, exhibiting pI 4.6 and pI 4.5, respectively, and the recovery of activity were about 1% in this condition.

Michaelis Constant

Michaelis constant of both enzymes for *p*-nitrophenyl phosphate was measured in the standard assay system. Michaelis constant of both intestinal and placental alkaline phosphatases was 0.3 mM.

Molecular Weight

The molecular weights of intestinal and placental alkaline phosphatase were determined according to the method of Whitaker[11] by gel filtration on Sephadex G-200. As shown in Fig. 2, the molecular weights were calculated to be 170000 for intestinal alkaline phosphatase and 130000 for placental alkaline phosphatase.

Optimum pH and pH Stability

Both intestinal and placental alkaline phosphatase had optimum pH of 10.5 in the standard assay system. The effect of pH on the stability of the enzyme was examined. As shown in Fig. 3, more than 80% of the original activity of intestinal alkaline phosphatase was retained at pH 5—11, under the condition of 0° for 1 hr. A similar result was obtained with placental alkaline phosphatase at pH 6—11. Intestinal alkaline phosphatase was rather stable in acidic condition, but not placental alkaline phosphatase.

11) J.R. Whitaker, *Anal. Chem.*, 35, 1950 (1963).

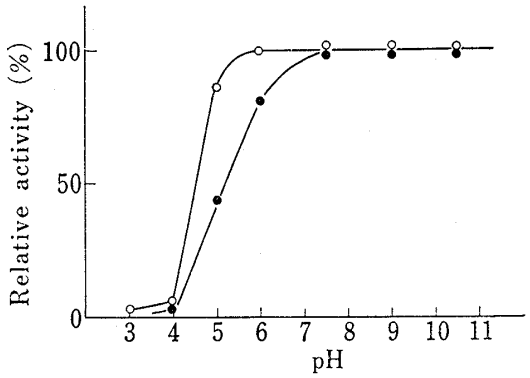

Fig. 3. Effect of pH on the Stability of Alkaline Phosphatases from Human Placenta and Small Intestininal Mucosa

The enzyme solution was treated at various pH for 1 hr at 0°.
—●—: placental alkaline phosphatase
—○—: intestinal alkaline phosphatase
pH 3—6: CH_3COOH-CH_3COONa buffer, pH 7—8: Tris-HCl buffer, pH 9—11: glycine-KCl-KOH buffer

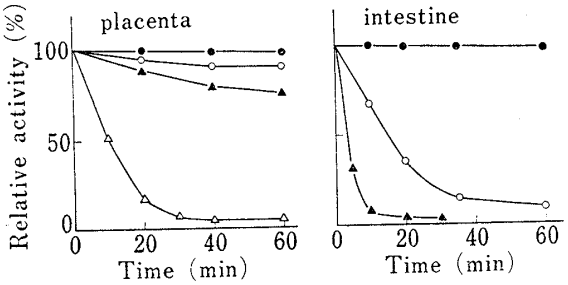

Fig. 4. Heat Stability of Alkaline Phosphatase from Human Placenta and Small Intestinal Mucosa

buffer: 0.1M glycine-KCl-KOH (pH 10.5)
—●—: 0°, —○—: 37°, —▲—: 50°, —△—: 60°

Effect of Temperature on the Activity and Heat Stability

Placental alkaline phosphatase exhibited a high activity at 45—55° in the standard assay system, but intestinal alkaline phosphatase showed the activity at 38°. As shown in Fig. 4, intestinal alkaline phosphatase completely lost its activity within 20 min at 50°. On the other hand, placental alkaline phosphatase was stable below 50°, and more than 75% of the maximum activity was observed after 60 min at 50°.

These results indicate that placental alkaline phosphatase is more stable than intestinal alkaline phosphatase. Clinically, intestinal alkaline phosphatase can now be differentiated from placental alkaline phosphatase based on their heat stability.

Effect of Metal Salts on the Activity

The solution of each alkaline phosphatase was preincubated with a metal salt at 37° for 30 min. After the mixture was diluted 10-fold with a buffer solution, the remaining activity was assayed. Both enzymes were strongly inhibited by 0.1 mM of $HgCl_2$ and $CdCl_2$, and activated by 0.1 mM of $MgCl_2$. Intestinal alkaline phosphatase was the same behavior of placental alkaline phosphatase against metal salts. These results suggest that Zn^{2+} might be contained in intestinal alkaline phosphatase as in placental alkaline phosphatase.

Effect of Chemicals on Alkaline Phosphatase Activity in the Enzyme System

The effect of some chemicals on the enzyme activity was examined. As shown in Table I, both enzymes were found to be markedly inactivated by metal chelating agents such as EDTA and *o*-phenanthroline, and by reducing agents such as 2-mercaptoethanol and sodium thioglycolate. Diisopropyl fluorophosphate did not show any effect on the alkaline phosphatase having active serine. The effect of Na_2HPO_4 on placental alkaline phosphatase activity was greater than that on intestinal alkaline phosphatase.

Effect of Various Compounds on the Activity in the Reaction System

Effect of amino acids on alkaline phosphatase activity was investigated in the standard assay system. As shown in Table II, behavior of intestinal and placental alkaline phosphatases against amino acids was identical, and both enzymes were sensitive to L-phenylalanine but not to homoarginine.

Placental alkaline phosphatase was markedly inhibited by 1 mM of PO_4^{3-}, but not intestinal alkaline phosphatase.

Tablb I. Effect of Chemicals on Alkaline Phosphatase Activity in the Enzyme System

Compound[a]	Relative activity (%)			
	Placenta concentration (M)		Intestine concentration (M)	
	10^{-3}	10^{-4}	10^{-3}	10^{-4}
None	100	100	100	100
DFP	73	88	3	113
EDTA	0	88	1	2
o-Phenanthroline	46	61	3	81
PCMB	68	81	87	100
NBS	83	84	88	113
Iodoacetic acid	62	76	24	100
KCN	81	88	5	106
Sodium thioglycolate	2	47	3	50
2-Mercaptoethanol	28	83	7	87
Sodium citrate	74	75	56	75
Na_2HPO_4	41	75	75	113

The enzyme was preincubated at 37° with various chemicals for 30 min (10 mM Tris-HCl, pH 7.4) and the activity was determined using *p*-nitrophenyl phosphate as a substrate in 0.1M glycine–KCl–KOH buffer, pH 10.5, at 37° for 30 min.

a) DFP=diisopropyl fluorophosphate, PCMB=*p*-chloromercuribenzoate, NBS=N-bromosuccinimide

Table II. Effect of Various Compounds on the Activity of Alkaline Phosphatase in the Reaction System

Compound	Relative activity (%)			
	Placenta concentration (M)		Intestine concentration (M)	
	10^{-3}	10^{-4}	10^{-3}	10^{-4}
Na_2HPO_4	34	80	105	102
Sodium citrate	85	99	87	98
L-Cysteine	57	94	45	97
L-Cystine	87	100	50	88
L-Phenylalanine	56	92	50	96
L-Tyrosine	90	95	96	102
L-Tryptophan	75	87	42	88
L-Histidine	88	98	91	105
Imidazole	95	98	103	107
Homoarginine	95	99	101	105

Discussion

Properties of purified human intestinal alkaline phosphatase was similar to those of purified human placental alkaline phosphatase with respect to isoelectric point, pH stability, Michaelis constant for *p*-nitrophenyl phosphate, and optimum pH. Molecular weight of placental alkaline phosphatase, as determined by Sephadex G-200 gel chromatography, was 130000 and was identical to that obtained by Howard, *et al.*[12] The molecular weight of intestinal alkaline phosphatase was 170000 and was larger than that of placental alkaline phosphatase.

In heat stability, intestinal alkaline phosphatase was different from placental alkaline phosphatase, being stable up to 38° and 65°, respectively. It is suggested that the difference in optimum temperature is due to the defference in heat stability.

12) H.S. Howard and A.J. Gottlieb, *Biochim. Biophys. Acta*, **194**, 170 (1969).

Effect of amino acid on intestinal alkaline phosphatase was similar to that on placental alkaline phosphatase and both enzymes are sensitive to 1 mM of L-phenylalanine, as described by Ghosh, *et al.*[13] However, L-homoarginine, a homolog of arginine and which is a strong inhibitor of bone and liver alkaline phosphatase,[14] showed virtually no effect on intestinal and placental alkaline phosphatases.

Placental alkaline phosphatase was markedly inhibited by 1 mM phosphate ion, but not intestinal alkaline phosphatase. The active site of the two alkaline phosphatases may be quite different.

Both alkaline phophatase were inhibited by metal chelating agents and by reducing agents, which fact suggests that metal ion and oxidized groups may be related to active enzyme forms.

13) N.K. Ghosh and W.H. Fishman, *J. Biol. Chem.*, **241**, 2516 (1966).
14) W.H. Fishman and Hsien-Gieh Sie, *Clin. Chim. Acta*, **29**, 339 (1970).