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Characterization of Tissue-specific Isozyme of Alkaline Phosphatase from Human Placenta and Intestine[1]

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Human alkaline phosphatases from placenta and intestine with molecular weight of 130000 and 170000, respectively, are composed of two subunits with molecular weights of approximately 65000 and 86000, respectively. These two enzymes were shown to have distinct different amino acid compositions and they are glycoprotein.

Isozyme about human alkaline phosphatase was also discussed.

Keywords——alkaline phosphatase isozyme; human placenta; human intestine; amino acid composition; sugar content

Alkaline phosphatase [E.C. 3.1.3.1] has been the subject of several recent studies relating to multiple electrophoretic forms of the activity. Multiple forms have been observed in rat, bovine and other species.[3] The activity of serum alkaline phosphatase is determined clinically for the purpose of diagnosis for liver and bone diseases, and it is often associated with cancer, namely Regan[4] and Nagao[5] isozymes which are thermostable. The fuction and the structure of Escherichia coli alkaline phosphatase was well studied by Schesinger and Barrett.[6] *Escherichia coli* alkaline phosphatase is a typical dimetric enzyme, and alkaline phosphatase from poricine and bovine intestine also show a typical property of dimetric enzyme. Although in recent years a considerable amount of work has been devoted to the molecular properties, the subunit composition, and to the catalytic mechamism of *Escherichia coli* alkaline phosphatase, little has been known concerning alkaline phosphatase from mammalian cells, especially from human tissues. In spite of the extensive studies on a number of mammalian enzymes, most data were obtained from partially purified enzyme preparations and the information concerning the subunit structure of human alkaline phosphatase was still lacking.

As for a series of studies on human alkaline phosphatates, we have previously reported about the purification of human alkaline phosphatases from placenta,[7] intestine,[8] bile,[9] liver,[10] and kidney.[11] In this paper, the compositions of amino acids and carbohydrates of alkaline phosphatases from human placenta and intestine were investigated and this two alkaline phosphatases were compared immunologically.

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3) H.N. Fernley, "The Enzymes," Vol. IV, Academic press, New York, 1971 p. 417.
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Materials and Methods

Enzymes——Alkaline phosphatases from human placenta and intestine were purified according to the method described in the previous papers.[7,8] The preparations obtained were homogeneous in the disc electrophoresis.

Amino Acid and Amino Sugar Analysis——According to the method of Liu and Chang,[12] the lyophilized alkaline phosphatase preparations (about 1 mg) were sealed and hydrolyzed *in vacuo* at 110° for 20 hr and 48 hr in 1 ml of 3 N toluenesulfonic acid containing tryptamine. The hydrolysates were neutralyzed with 1 N NaOH and analyzed with a JEOL Amino Acid Autoanalyzer Model JLC-6AH.

SDS-electrophoresis——Polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate was carried out according to the method of Weber and Osborn[13] with a minor modification. Proteins were prepared for electrophoresis by denaturation in 10 mM sodium phosphate (pH 7.2) containing 1% sodium dodecylsulfate with 1% 2-mercaptoethanol and 8 M urea. Samples were incubated at 37° for 4 hr and then processed as follows: 10 μg of each protein was applied to 5% polyacrylamide gel. Electrophoresis run (8 mA/tube) lasted 4 hr at room temperature in 0.1 M sodium phosphate buffer (pH 7.2) and 0.1% sodium dodecylsulfate. Proteins were stained with amido black 10B.

Clarification of Glycoprotein——After running polyacrylamide disc electrophoresis by the method of Davis,[14] glycoprotein in the gel was treated with periodic acid followed by staining with fuchsin-sulfate.[15] The glycoprotein was also stained with amido black 10B.

Staining of Enzyme Activity——Enzyme activity on polyacrylamide gel was stained by the method of Smith, *et al.*[16]

Determination of Neutral Sugar and Sialic Acid——Neutral sugar and sialic acid were determined by the method of Sweeley and Walker.[17] A JEOL, Model JGC-20KPF, gas chromatograph equipped with a hydrogen flame ionization detector was used and the 2 m×2 mm I.D. glass column was packed with 3% OV-1 on Gas-Chrom Z (80—100 mesh).

Antiserum for Alkaline Phosphatases from Human Placenta and Intestine——Antiserums were prepared according to the method described in the previous papers.[7,8]

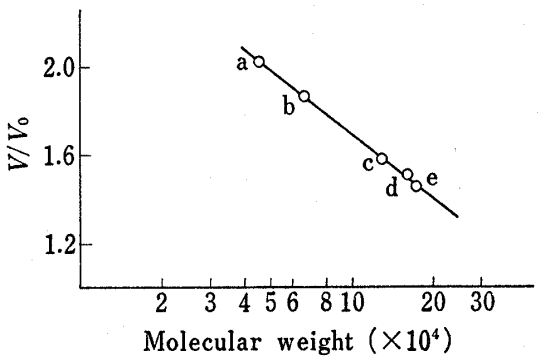

Fig. 1. Determination of the Molecular Weight of Alkaline Phosphatase by Gel Filtration of Sephadex G-200

a, ovalbumin; b, bovine serum albumin; c, placental alkaline phosphatase; d, γ-globulin; e, intestinal alkaline phosphatase.

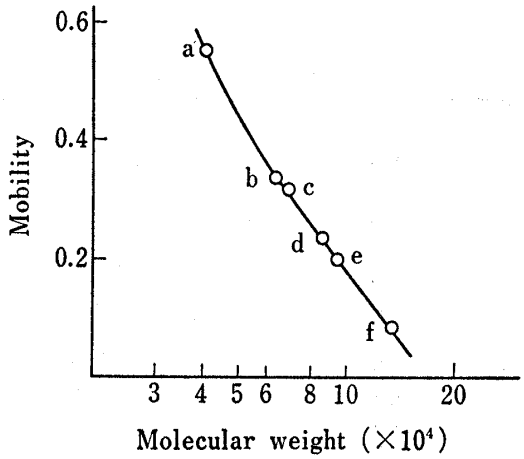

Fig. 2. SDS Electrophoresis of Human Placental and Intestinal Alkaline Phosphatase

5% polyacrylamide gel was used and protein was stained with Amido Black 10B. For experimental details see text.
a, ovalbumin; b, placental alkaline phosphatase; c, bovine serum albumin; d, intestinal alkaline phosphatase; e, phosphorylase a; f, β-galactosidase.

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Results

Molecular Weight

Molecular weights of alkaline phosphatases from human placenta and intestine were investigated by the use of Sephadex G-200 gel chromatography as shown in Fig. 1. It was found that molecular weights of placental and intestinal alkaline phosphatases were 130000 and 170000, respectively. Molecular weight of placental alkaline phosphatase was smaller than that of intestinal alkaline phosphatase and agreed with the value calculated by Harkness.[18] Molecular weights of subunits of alkaline phosphatases were investigated by the method of Weber and Osborn[13] and the results are shown in Fig. 2. A subunit molecular weight of the placental alkaline phosphatase was calculated 65000 and that of the intestinal alkaline phosphatase was 86000. From these results, it was found that placental and intestinal alkaline phosphatases were composed of 2 subunits, namely dimer.

Compositions of Amino Acids and Amino Sugars of Alkaline Phosphatase

Amino acids and amino sugars of the alkaline phosphatase were analyzed by the method of Liu and Chang[12] and the result is shown in Table I. Total acidic amino acids of the alkaline phosphatases were larger than that of the total amino acids and these results agreed with the isoelectric points of both enzymes of pI 4.5 and pI 4.6, respectively.[19]

Table I. Amino Acid and Amino Sugar Compositions of Human Placental and Intestinal Alkaline Phosphatase

Amino acid and amino sugar	No. of residues of amino acid and amino sugar			
	Placental alkaline phosphatase		Intestinal alkaline phosphatase	
	20 hr Hydrolysate	48 hr Hydrolysate	20 hr Hydrolysate	48 hr Hydrolusate
Trp.	29	26	76	68
Lys.	47	50	21	21
His.	26	29	10	11
Arg.	71	73	20	21
Asp.	113	111	107	107
Thr.	79	79	155	157
Ser.	68	64	157	143
Glu.	118	117	139	141
Pro.	74	72	148	146
Gly.	104	103	121	122
Ala.	121	121	104	104
Cys.	6	6	20	20
Val.	50	59	77	94
Met.	27	26	16	16
Ileu.	26	33	29	36
Leu.	87	88	108	112
Tyr.	40	39	46	47
Phe.	38	38	48	50
Glucosamine	33	27	69	62
Galactosamine	5	5	50	45

Protein was hydrolyzed with 3N toluenesulfonic acid as described under Materials and Methods. No. of residues was calculated as mol/130000 *g* and mol/170000 *g* of amino acid and amino sugar for placental and intestinal alkaline phosphatases, respectively.

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Composition of each amino acid of the placental alkaline phosphatase was different from that of the intestinal alkaline phosphatase and it was indicated that the primary structures of the two alkaline phosphatases were different from each other and it seemed that the genetic control for alkaline phosphatases was different from that for originated tissues. Amino sugar contents of both enzymes were also investigated like amino acids. Amino sugars were contained in both enzymes, and the amino sugar content of the intestinal alkaline phosphatase was larger than that of the placental alkaline phosphatase.

Sugar Content of the Two Alkaline Phosphatases

Figure 3 indicates the results of the staining of protein and sugar of the two alkaline phosphatases on 7.5% polyacrylamide gel, and it was found that placental and intestinal alkaline phosphatases were glycoprotein. By the treatment with neuraminidase the placental enzyme varied its mobility on the zymogram, as shown in Fig. 4, but not the intestinal alkaline phosphatase. From this result, it seems that sialic acid is contained in the placental alkaline phosphatase but not in the intestinal alkaline phosphatase. Futhermore, sugar contents of the enzymes were analyzed by the use of gas chromatography to clarify the above results in detail. The result is shown in Table II. Sugar content of the placental alkaline phosphatase was smaller than that of the intestinal alkaline phosphatase. In particular,

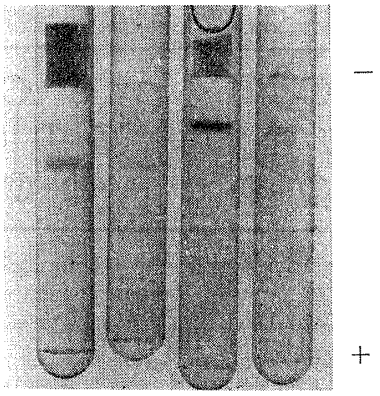

Fig. 3. Clarification of Glycoprotein

Staining of protein (a) and sugar (b) of placental alkaline phosphatase, and of protein (c) and sugar (d) of intestinal alkaline phosphatase. 7.5% polyacrylamide gel was used and the disc electrophoresis was carried out at 4° for 70 min.

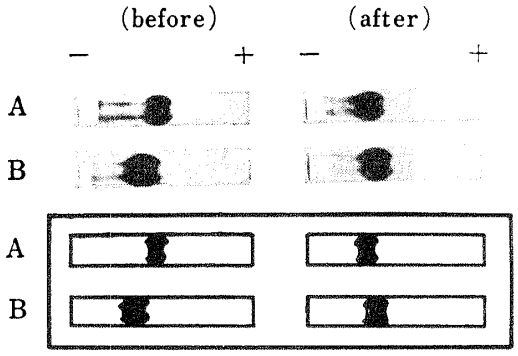

Fig. 4. Effect of Neuraminidase on Alkaline Phosphatase from Human Placenta and Intestine

Alkaline phosphatases and neuraminidase (0.6 U) were incubated at 37° for 20 hr, and then the reaction mixture was applied to electrophoresis. 5% polyacrylamide gel was used and staining was carried out using naphylphosphate-Fast Blue BB.
A: alkaline phosphatase from human placenta before or after neuraminidase treatment, B: alkaline phosphatase from human intestine before or after neuraminidase treatment.

TABLE II. Quantity of Monosaccharide in Human Placental and Intestinal Alkaline Phosphatases

Carbohydrate residue	Placental alkaline phosphatase		Intestinal alkaline phosphatase	
	(mol/mg)	(mol/130000 g)	(mol/mg)	(mol/170000 g)
Glucose	0.009	1.2	trace	trace
Galactose	0.292	38.0	0.754	128.2
Mannose	0.102	13.3	0.376	64.0
Fucose	0.022	2.9	0.357	60.7
Sialic acid	0.217	28.2	N.D[a)]	N.D[a)]

a) Not detectable.

the sialic acid contents of these two enzymes differ from each other. The effect of neuraminidase on these two enzymes were compatible with their sialic acid contents and it was found that the migration of the mobility of placental enzyme was due to its sialic acid content.

Immunological Properties of These Two Enzymes

Cross reaction for these two enzymes was investigated by means of radioimmunoassay system for human placental and intestinal alkaline phosphatase as previously described.[7,20] The results are shown in Fig. 5 and it was found that at a high concentration of antiserum, cross reaction to each other was observed. But these phenomena were not recognized at the concentration of diluted antiserum (10000—15000×) used in radioimmunoassay system. Consequently, the presence of a common antibody in the antibody in the antiserums is presumed and examined by the method of absorption of antiserum. Antiserum of human placental alkaline phosphatase was absorbed by human intestinal alkaline phosphatase and *vice versa*. Figure 6 indicates that there is a characteristic antibody in each antiserum.

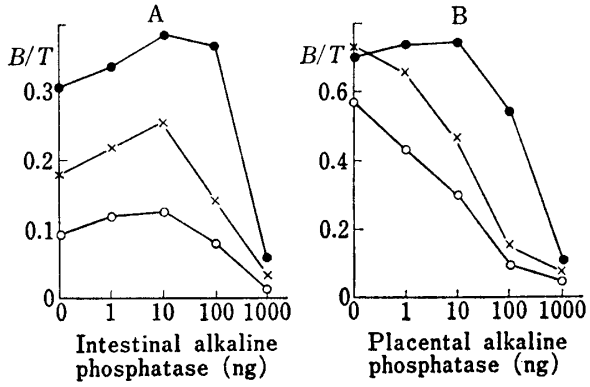

Fig. 5. Cross Reaction between Human Placental and Intestinal Alkaline Phosphatases

A: It shows dose response curve of intestinal alkaline phosphatase in radioimmunoassay system for intestinal alkaline phosphatase using diluted anti-placental alkaline phosphatase antibody in place of anti-intestinal alkaline phosphatase antibody (100×, 500×, 1000×).

B: It shows dose response curve of placental alkaline phosphatase in radioimmunoassay system for placental alkaline phosphatase using diluted anti-intestinal alkaline phosphatase antibody in place of anti-placental alkaline phosphatase antibody (100×, 500×, 1000×).

●, 100×; ×, 500×; ○, 1000×.

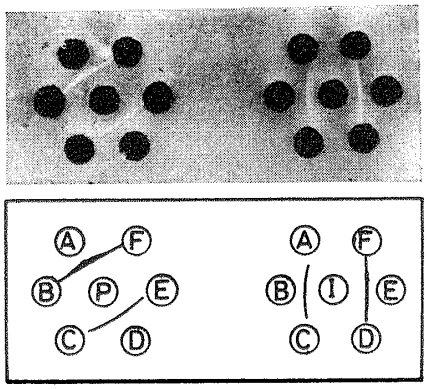

Fig. 6. Micro-ouchterlony Immunodiffusion Patterns of Human Placental and Intestinal Alkaline Phosphatases

Anti-placental alkaline phosphatase antibody was absorbed by intestinal alkaline phosphatase and *vice versa*.

A,D: purified human placental alkaline phosphatase, B,E: purified human intestinal alkaline phosphatatse, C,F: partially purified human liver alkaline phosphatase, P: antiserum of placental alkaline phosphatase, I: antiserum of intestinal alkaline phosphatase.

Discussion

Human alkaline phosphatases from placenta and intestine contain neutral sugars and amino sugars like other mammalian alkaline phosphatases. Neutral sugar content in the placental alkaline phosphatase was somewhat larger than that described by Ghosh, *et al.*[21] These differences would be due to the hydrolytic condition of the enzyme. The condition we used was more mitigative than that used by them. Sialic acids in the intestinal alkaline phosphatase were not detectable, and this result agreed with that obtained in rat intestinal alkaline phosphatase described by Komoda, *et al.*[22] These data explain that the

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treatment with neuraminidase has no effect on the electrophoretical mobility of the intestinal lakaline phosphatase. Futhermore, it was concluded that human placental and intestinal alkaline phosphatases were composed of 2 subunits of equal or very similar weight like alkaline phosphatases from porcine kidney,[23] bovine kidney,[24] calf intestine[25] and *Escherichia coli*,[6] when the subunit molecular weights of human placental and intestinal alkaline phosphatases were determined by SDS electrophoresis. The subunit molecular weight of placental alkaline phosphatase was 65000, which was larger than that of 58000, described by Gottlieb and Sussman[26] and smaller than that of human intestinal alkaline phosphatase of 86000. In addition, the subunit molecular weight of human intestinal alkaline phosphatase was larger than those of alkaline phosphatases from calf intestine[25] and rat liver,[27] but it was very similar to pig and bovine kidney alkaline phosphatases.[23,24]

Both enzymes were found to be acidic proteins by comparing the content of acidic amino acid with that of basic one on the basis of amino acid analysis. This conclusion was also confirmed by the determination of isoelectric point. There is a distinct difference in amino acid compositions between two enzymes. On the basis of these results, it was anticipated that the primary structures between placental and intestinal alkaline phosphatases may be different and both enzymes may be synthesized by the control of different genes. These differences also indicated that these two enzymes were differentiated immunologically.

Although the unique physiological function of each isozyme remains to be elucidated, the present study has shown that human alkaline phosphatase is truly isozyme and that this isozyme exhibits a tissue-specific distribution.

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