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BLOOD APPEARANCE OF RAT ALKALINE PHOSPHATASE ORIGINATING FROM THE DUODENUM IN VITRO

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SUMMARY

The major source of rat serum alkaline phosphatase (ALP) is well known to be from the intestinal enzyme, but it is still unclear whether it is from the duodenal or the ileal enzyme. The organic origin was investigated by means of two-dimensional electrophoresis. Major isoelectric points and molecular masses for activities of duodenal enzyme treated with both phosphatidylinositol-specific phospholipase C and neuraminidase were identified apparently with those of the major serum enzyme. In organ culture, the normal duodenal enzyme was released in the highest amounts to the culture medium. These results indicate that the major source of serum ALP in adult rats is basically from the duodenal enzyme. On the other hand, lectin affinity chromatography for ALPs showed that the ALP in the medium from culture duodenum and liver had the same complex-type sugar chain as with the ALP in the duodenal tissue. Although the duodenal ALP induced by glucosamine in vitro had the hybrid-type chain, sugar chains of the induced ALP in the culture medium were of the complex type, indicating that medial ALPs possessing the same sugar chain as the native duodenal enzyme, complex type, are mainly released from their tissues in normal conditions.

INTRODUCTION

The source of serum alkaline phosphatase (ALP) has been investigated with various animal species. The ALP activity in rat serum is well known to be higher than that of other mammalian species, and to be further increased after fat feed-

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ing in rats [1, 2]. Saini and Posen [3] have reported that anti-rat intestinal ALP antibody does not react with serum ALP in fasted rats but does so in fed rats which indicates that the intestinal ALP is the major source of serum ALP in fed rats. On the other hand, the basal level of rat liver ALP is very low, but the liver enzyme activity is induced easily by several treatments [4], e.g. , bile-duct ligation, partial hepatectomy, cholera toxin and glucocorticoid. Moreover, the elevation of the tissue-unspecific type ALP activity is usually accompanied by that of the serum enzyme activity. Hence the organic origin of rat serum ALP is not only derived from the intestine but also the tissue-unspecific type as a minor constituent.

In the rat intestine, there are at least two forms of ALPs, membranous and soluble. Not the membranous but soluble form enzymes are generally detected in their serum. Young et al. [5] reported that the soluble form in ileal ALP might be major source of the serum enzyme in the suckling rat. The present data for normal adult rats seemed to show that the major source of serum ALP is a soluble form of duodenal ALP. Further, another purpose of this work was to confirm that a serial lectin affinity technique described previously by us [6, 7] is useful for the assessment of sugar chains of ALPs with the enzyme-active form.

EXPERIMENTAL

Enzyme preparations and enzyme assay

Male Wistar rats, 30 days old, purchased from Shizuoka Experimental Animals, Japan, were used for in vivo and in vitro experiments. Liver, bone (calvarium), kidney, duodenum and ileum were retained as the organic origin of ALPs. Each sample removed was homogenized in 10 m*M* Tris–HCl buffer (pH 7.5) containing 0.5% Triton X-100, 1 m*M* benzamidine chloride and 0.3 m*M* phenylmethylsulphonyl fluoride (PMSF). The homogenates were centrifuged at 15 000 *g* for 15 min; the resulting supernatant was then treated with 20% *n*-butanol for 30 min to remove lipid residues in the crude enzyme solution, and concentrated with 60% acetone.

The specific activity of ALP isosymes in the various tissues were measured by the release of *p*-nitrophenol from disodium *p*-nitrophenyl phosphate at 405 nm, before the treatment of samples with *n*-butanol and acetone. The assay was performed with 50 m*M* carbonate–hydrogencarbonate buffer (pH 10.0) containing 5 m*M* magnesium chloride, at 37 °C as described previously [8]. Protein concentrations were determined according to Lowry et al. [9] with bovine serum albumin as a standard.

Two-dimensional electrophoresis of ALPs

The preparation for electrophoresis was further treated with 1 mU/ml of phosphatidylinositol (PI)-specific phospholipase C (PLase C), which was obtained from *Bacillus thuringiensis* [10], in order to remove 1,2-diacylglycerol residues of the PI moiety in ALP molecules, and further digested with 100 mU/ml of neuraminidase (Nakarai Chemical, Japan) to eliminate the sialic acids [11]. According to the method of McKenna et al. [12], the resulting ALP preparation was first subjected to isoelectric focusing in 4.5% polyacrylamide gel (1×35 mm)

containing 5.4% Ampholine (pH 3.5–10+pH 3.5–5, 4:1). After pre-loading for 20 min at 50 V, the preparation in the gels was focused for 30 min at 300 V. The focused gels were further treated in the other dimension in a gradient polyacryl amide gel slab (4–17%) containing 0.1% sodium dodecylsulphate (SDS). After the preparations had been run at 300 V per slab for 1 h, the gels were soaked in a solution of 5-bromo-3-indolylphosphate *p*-toluidine salt (BIP, 2 mg/ml) (Wako, Japan) in 1.0 *M* ammediol–HCl buffer (pH 10.2), supplemented with 1 m*M* magnesium chloride and 10 μM zinc chloride, and were stained for ALP activities. Electrofocusing gels in one-dimensional microtubing were also stained with BIP.

Organ culture

The tissues of rat liver, kidney, calvarium, duodenum and ileum were used for organ culture. The liver and duodenum from rats, bile-duct ligated (BDL) [13] or partially hepatectomized [14] to induce ALP activity, were also subjected to organ culture. The medium consisted of DM-170 (Kyokuto Pharmaceutical Industrial, Japan) containing 50 U/ml of penicillin G and streptomycin, 1 m*M* benzamidine chloride and 0.3 m*M* PMSF. The organ culture procedure was carried out as described previously [6, 15]. Briefly, nine pieces of tissue (1 mm^3) were placed in each chamber on a wet Millipore filter (SS type and pore size 3 μm). Two chambers were then placed in 7.0 ml of medium with or without various supplements in a roller tube (120×35 mm) and cultured at 37°C in an atmosphere of carbon dioxide–air (1:19). After cultivation, the explants were homogenized, and the incubating media were centrifuged at 105 000 *g* for 1 h to remove the tissue debris and to obtain the soluble fraction of ALPs. Both the homogenate and the medium were then treated with *n*-butanol and acetone.

Lectin affinity chromatography

The lectins used were concanavalin A (*Canavalia ensiformis*) (Con A) purchased from Pharmacia, Sweden, wheat germ agglutinin (*Triticum valgaris*) (WGA) and phytohemagglutinin-E (*Phaseolus vulgaris*) (PHA-E) from E.Y. Labs., U.S.A.

Lectin affinity chromatography was performed as described previously [6, 7]. Briefly, an enzyme preparation was applied to the Con A column (10×0.5 cm I.D.) and three fractions were then obtained by using two different concentrations (10 and 500 m*M*) of α-methyl-D-mannoside (αMM): an unbound fraction (fraction I), a weakly bound fraction (fraction II) and a strongly bound fraction (fraction III). Fractions I and III were further applied to the PHA-E and WGA columns, respectively. The unbound and bound fractions were separated on the respective columns by using 0.1 *M* N-acetyl-D-glucosamine as an elution buffer.

RESULTS

Two-dimensional electrophoresis for ALPs in various organs

For ALPs as membrane-bound glycoproteins it has been shown that membranous PI is involved in the attachment of ALPs to the biomembrane [16]. We found previously that ALPs were released specifically from membranes by treat-

TABLE I

ISOELECTRIC POINTS FOR RAT ORGAN ALKALINE PHOSPHATASE ISOZYME

Each value was obtained from the results of isoelectric focusing for ALP preparations. The italicized numbers show major bands for the enzyme activity stain.

Organ	Enzymatically active bands for alkaline phosphatases																
Serum	*3.9*		4.2	4.3		4.9				6.4	*6.45*		6.6	*6.9*			
Duodenum								*5.6*			*6.45*	6.5		*6.9*	7.4		
Ileum		*4.0*									*6.45*						
Bone	*3.9*			4.3		*4.9*	*5.2*										
Kidney					4.4	*4.9*	*5.2*	5.6									
Liver	*3.9*					*4.9*											
Duodenal culture★																	
Tissue								*5.6*			*6.45*	6.5		*6.9*	7.4	8.3	
Medium									6.1		*6.45*		*6.6*	*6.9*	*7.4*	8.3	8.55

★The duodenum was cultured for 24 h, and ALPs in the resulting tissue and medium were subjected to isoelectric focusing analysis.

ment with PI-specific PLase C [10], suggesting that the present enzyme preparations would contain PI molecule(s) and the electrophoretic mobilities might be affected by the presence of PI. The preparations for electrophoresis were then treated with PI-specific PLase C. Apparent isoelectric points (p*I*) for respective isozymes are given in Table I. Two major identical p*I*s of 6.45 and 6.9 for part of the serum ALP were detected in the duodenal enzyme. The major p*I*s of liver, bone and kidney ALPs were separated clearly from those of the duodenal enzyme. The p*I*s of all enzymes treated with PI-specific PLase C differed considerably from those of the non-treated enzymes (data not shown), which indicates that it is essential in the measurement of p*I*s of ALPs to treat the enzyme preparation with both PI-specific PLase C and neuraminidase.

The similarity between the duodenal and serum ALP was further clarified by two-dimensional electrophoresis as shown in Fig. 1. Molecular masses of 142 000 of p*I*s 6.45 and 6.9 in the major serum enzyme were identified with the part of the duodenal enzyme. The molecular mass of p*I* 4.0 in the ileal enzyme was found to be 130 000, and that of p*I* 3.9 in the serum enzyme was 162 000.

Rate of release of the ALP activity from the duodenum into the medium

By means of organ culture, release of ALP activity in the medium was studied. The ALP activities in five tissues subjected to the organ culture were as follows: liver, 17; kidney, 390; calvarium, 60; duodenum, 880; and ileum, 42 nmol/min per explant. Enzyme activities in the medium were expressed as a percentage of the activities in the medium per medium plus tissue, and results are shown in Fig. 2. After incubation for 24 h, the enzyme activities released from the ileum, liver, kidney and calvarium were all within 6% (Fig. 2b). In contrast, that from the duodenum accounted for 17%, and the activity released from the duodenum increased with time up to 48 h (Fig. 2a). The specific activity in the duodenal tissue was unchanged during the culture (Fig. 2a), and further there was no change

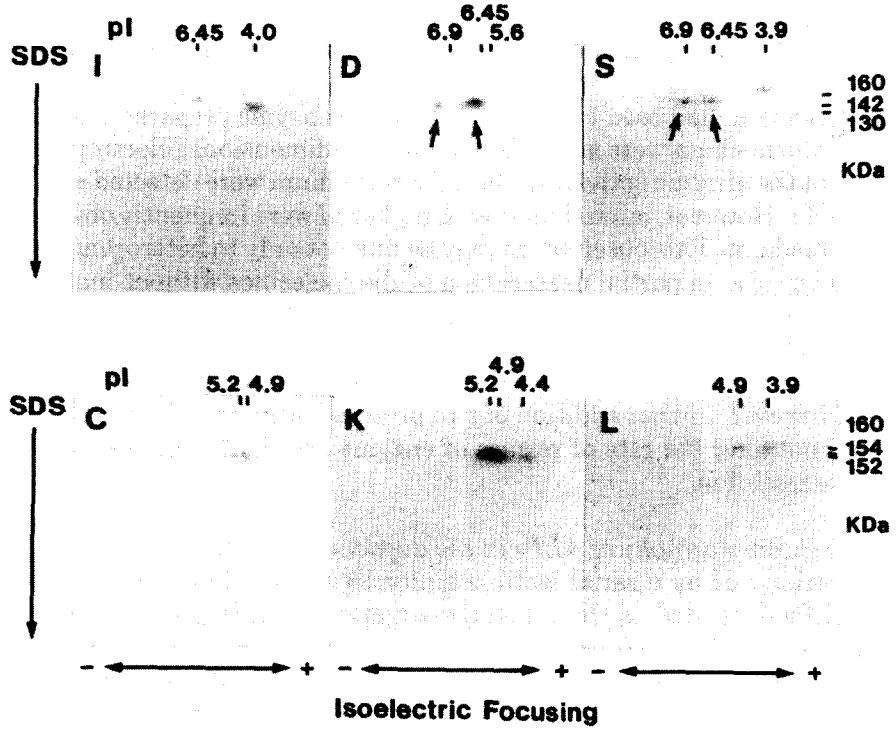

Fig. 1. Two-dimensional electrophoresis of ALPs from various rat organs. Focused gels were subjected to two-dimensional gradient polyacrylamide gel electrophoresis (PAGE) as described under Experimental. Isoelectric focusing was run to the horizontal axis and SDS-PAGE to the vertical axis. Symbols: S, serum; D, duodenum; I, ileum; L, liver; K, kidney; and C, calvarium. kDa = kilodaltons.

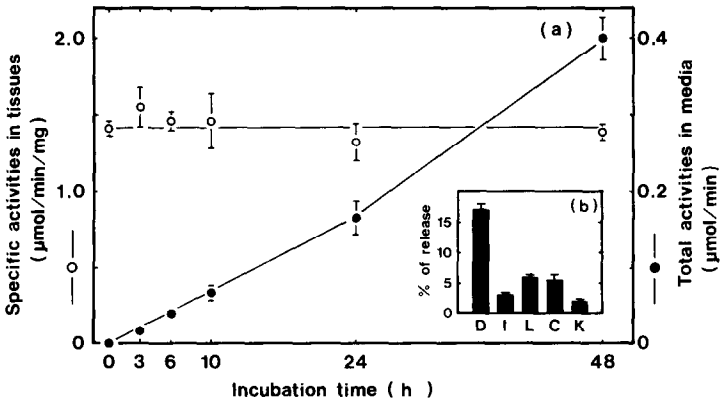

Fig. 2. Release of ALP activity from the duodenum into medium on the organ culture. Explants from (a) the duodenum and (b) various organs (symbols as in Fig. 1) were cultured as described under Experimental. Each value represents the mean ± standard deviation (the bar on the symbol) of three experiments.

in the total enzyme activities in tissues and media from 10 to 48 h cultures (data not shown), which suggests that the enzyme would be inactivated within 48 h in vitro.

After the duodenum had been cultured for 24 h, the enzyme preparations from the tissue and the medium were also subjected to two-dimensional electrophoresis. Major p*I*s of the enzyme activity in the culture medium were detected at 6.45 and 6.9 (Table I). However, minor bands with higher p*I* were frequently observed in the culture medium. This observation may be due not only to heterogeneity of the molecules but also to partial degradation of the molecules without inactivation of enzyme during culture for 24 h. In the present culture, PMSF and benzamidine were added to the medium. The percentage of ALP released from tissues was increased significantly in the absence of these two protease inhibitors (data not shown). However, further addition of two protease inhibitors, leupeptin and pepstatin, did not alter the rate of release of enzyme activity in the presence of PMSF and benzamidine.

Con A affinity chromatography of ALPs in the duodenum in vitro

We demonstrated using a serial lectin affinity technique that lectin-binding patterns of ALPs depended on the respective organs, indicating that the lectin-binding specificity of the sugar chains in the tested ALPs is a useful marker for the nature of their organic origin [6, 7]. In preliminary experiments, the serum from fasted and fed rats, which was treated with *n*-butanol and acetone, was first subjected to lectin affinity chromatography. As reported previously, Con A binding patterns of serum ALP from fasted rats [6] were similar to those of the bone type. However, the patterns of serum ALP from fed rats which exhibited higher ALP activities were those of intestine rather than bone. This observation is consistent with earlier reports [1–3]. On the other hand, we have also validated that the patterns of human serum ALP were identical with those of the liver and/or bone type. In this study, this technique was applied to the identification of ALPs in media and tissues. The ALP preparations (see Fig. 2a) were first assessed by lectin affinity chromatography (Fig. 3). The percentage recoveries on the Con A column for the ALPs were 85–100%. Fractions I and III obtained from the Con A column were bound nearly 100% to the PHA-E column and the WGA column, respectively. The high affinity of fraction I to the PHA-E column indicates that the ALP in fraction I possesses more branched or bisecting complex sugar chains [6]. On the other hand, the high affinity of fraction III to the WGA column indicates that this fraction is classified as hybrid-type chains [6]. Enzyme activities in the chromatographic study were expressed as the relative activity (activities of each tube per total tubes). As shown in Fig. 3a, the relative activities of fraction III in the tissue on the Con A column increased in a time-dependent manner. On the other hand, the Con A binding pattern of ALPs in the medium remained unchanged consistently for 48 h (Fig. 3b), and was similar to that of the ALP in the uncultured duodenum but not to that in the ileum [6].

Swainsonine is a potent, reversible inhibitor of lysosomal α-mannosidase II and also Golgi α-mannosidase II [17]. In this study, after incubation of the duodenum with swainsonine, the sugar chain(s) of ALPs in the medium were assessed.

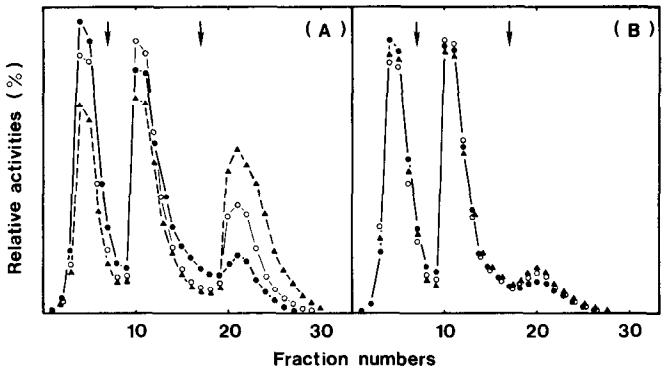

Fig. 3. Con A affinity chromatography of ALPs in the duodenum cultured for 3, 24 and 48 h. A and B indicate elution profiles of ALP in the tissue and medium, respectively. After washing with the buffer, two fractions were eluted with 10 and 500 m*M* αMM (indicated by the arrow). ●, 3; ○, 24; and ▲, 48 h.

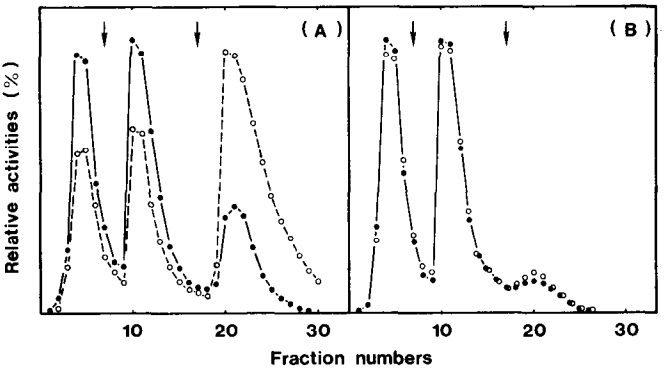

Fig. 4. Con A affinity chromatography of duodenal ALP treated with swainsonine in vitro. The duodenum was cultured for 24 h with (○) or without (●) 1.6 μ*M* swainsonine and ALPs in the resulting tissue and medium were passed to the Con A column. A and B indicate the elution profiles of ALPs in the tissues and media, respectively. Arrows indicate the starting points of 10 and 500 m*M* αMM, respectively.

As shown in Fig. 4, the relative activity of fraction III on the Con A column increased in the tissues supplemented with swainsonine, as expected. Those in the medium were identical with the control without swainsonine.

To induce ALP activity, glucosamine was added to a culture medium of duodenum (Table II); the specific activity in the tissue increased 1.5-fold, as expected. However, no increase in the total enzyme activity in the medium was detected. Consequently, the percentage of released ALP activity was diminished significantly (Table II). The Con A binding pattern of ALPs in media and tissues treated with glucosamine was similar to that in the presence of swainsonine (data not shown), that is, the relative activity of fraction III in the tissue was increased, whereas that in the medium was not increased.

TABLE II

EFFECTS OF SUPPLEMENTS ON THE DUODENAL ALP ACTIVITY

The duodenum was cultured for 24 h with or without 1.6 μM swainsonine or 10 mM glucosamine. Each value is the mean ± standard deviation of three experiments.

	Specific activity of tissues (nmol/min·mg)	Total activity (nmol/min) in		Percentage release (M/M+T)
		Tissues (T)	Media (M)	
Control	932 ± 144	517 ± 65	106 ± 16.7	17.0 ± 1.1
Swainsonine	965 ± 33	609 ± 56	131 ± 1.4	18.7 ± 1.2
Glucosamine	1651 ± 54★	882 ± 29★	84 ± 10.8	8.6 ± 1.0★

★$P < 0.05$ (when compared with the control by Student's t-test).

Effects of BDL and partial hepatectomy on the sugar chain(s) in liver and duodenal ALPs

We demonstrated previously that ALP activities not only in rat liver but also in the intestine were induced by BDL or partial hepatectomy [6, 15]. At 24 h after BDL or partial hepatectomy, the liver and the duodenum removed from sham-operated and operated rats were further cultured for 24 h (Table III). In the induced ALP, the percentage of ALP released from the liver in the BDL rat was increased, whereas that from the hepatectomized rat was low or zero. The percentage of released ALP in the duodenum from the BDL or the hepatectomized rat was unchanged in comparison with the sham-operated rat. These ALP preparations were then applied to the Con A column. The Con A binding pattern of the duodenum from the BDL-rat was similar to that in the presence of swain-

TABLE III

EFFECTS OF ALP INDUCTION IN VIVO ON THE PERCENTAGE OF ALP RELEASE IN VITRO

The tissues of the differently operated rats were cultured for 24 h. Each value represents the mean ± standard deviation of three experiments.

	Specific activity of tissues (nmol/min·mg)	Total activity (nmol/min) in		Percentage release (M/M+T)
		Tissues (T)	Media (M)	
Duodenum				
Sham operated	988 ± 94	540 ± 79	110 ± 11.0	15.7 ± 1.0
Bile-duct ligation	534 ± 65	341 ± 34	63 ± 13.3	15.5 ± 1.5
Partial hepatectomy	1137 ± 140	667 ± 50	131 ± 23.3	16.3 ± 1.4
Liver				
Sham operated	24 ± 2.3	40 ± 5	2.5 ± 0.5	5.7 ± 0.5
Bile-duct ligation	127 ± 11.8★	166 ± 15	23.2 ± 3.4	12.2 ± 1.2★
Partial hepatectomy	95 ± 7.1★	133 ± 13	7.4 ± 1.4	5.2 ± 0.4

★$P > 0.05$ (when compared with the sham-operated rat by Student's t-test).

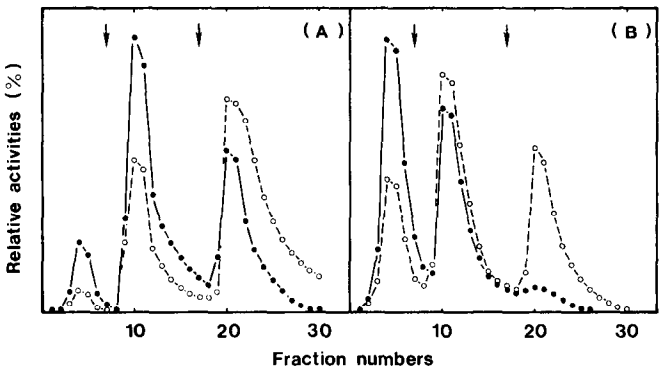

Fig. 5. Con A affinity chromatography of liver ALPs in sham-operated and BDL rats. The liver of sham-operated (●) and BDL (○) rats were cultured for 24 h and ALPs in the resulting tissue and medium were passed to the Con A column. A and B indicate the elution profiles of ALPs in the tissue and media, respectively. Arrows indicate the starting points of 10 and 500 m*M* αMM, respectively.

sonine or glucosamine in vitro (data not shown). However, that of the liver ALP exhibited unique patterns (Fig. 5). Although the relative activity of fraction III in the liver from the sham-operated rat accounted for about 40%, those in the medium in the sham-operated rat were hardly detected, and similar to those in the duodenal tissue and the medium. However, when liver from the BDL rat was cultured, the activity of fraction III in both tissue and medium were increased in comparison with the sham-operated rat. On the other hand, no increase in the activity of fraction III was observed in the cultured medium of the liver from the partial hepatectomized rat or the sham-operated rat.

DISCUSSION

The first purpose of this work was to investigate the major organic origin of rat serum ALP by means of two-dimensional electrophoresis. In rats, the problem is to establish whether its origin is the duodenum or ileum. We treated the ALP preparation with PI-specific PLase C before isoelectric point measurements. Membranous PI is known to be involved in the attachment of ALPs to biomembranes [16], and ALPs are released specifically from membranes by treatment with PI-specific PLase C [10]. Consequently, partially purified ALP preparations would contain PI molecules, and it is expected that the charge of these ALPs might be altered by PI. There is a report that PLase Cs originating from *S. aureus*, *B. cereus* and *Cl. welchii* only release the liver/bone/kidney-type ALP isozyme and not the intestinal-type enzyme [18]. However, it is not known whether PLase Cs exhibit PI-specific PLase C, but it is reported that treatment of ALPs with PLase C (*B. cereus*, *Cl. perfringens*) altered the mobilities of native ALPs in electrophoresis [19]. In fact, we observed that the p*I*s of the intestinal ALP were obviously changed by treatment with the PI-specific PLase C from *B. thuringiensis*. Accordingly, it is essential for the measurement of the p*I*s of ALPs to treat the enzyme preparation not only with neuraminidase but also with PI-specific PLase C. Measurement of the isoelectric points of ALPs showed that serum ALP

had two major p*I*s of 6.45 and 6.9. These two p*I*s were detected in the duodenal ALP but not in the ileal enzyme. Two-dimensional electrophoresis further clarified the similarity between the serum and the duodenal ALP.

These results confirmed that the major serum ALP in the adult rat is derived from the duodenum. It has been reported that the ileal ALP in the suckling rat is a major source of the serum enzyme [5]. However, substrate specificity of intestinal ALP in the adult rat is different from that in the suckling rat [20]. Further, Besman and Coleman [21] have recently reported evidence for the difference in the N-terminal amino acid sequence between calf and bovine intestinal ALPs, indicating that the primary structure, including the C-terminal hydrophobic region, differs between ALPs of adult and suckling rats. Changes in the protein moieties as one of the translocation mechanisms into blood may alter the organic origin in serum ALPs between suckling and adult rats.

Although most intestinal ALP molecules in vivo are generally localized on biomembranes at the brush border, the intestinal ALP in serum has a soluble and non-polymeric character. In this study, an organ culture system was applied to in vitro experiments on the release of ALPs from explants. The duodenal ALP was found to be released in the greatest amounts into the medium in comparison with the ileal enzyme. It must be given careful consideration that localization in the villus differs between duodenal and ileal ALPs: the duodenal ALP exists at the tip of the brush border whereas ilial ALP exists at the deep region of the cells [22] and, further, both the release of ALP into the medium in vitro and the secretion of ALP into blood in vivo cannot be discussed on the same basis. However, the large amounts of duodenal ALP in the medium are in agreement with the results of isoelectric focusing. Moreover, ALPs in the culture medium of the duodenum had the same two major p*I*s as the part in the serum. However, ALPs in the medium also had higher p*I* values. The rate of release of ALPs was often enhanced in the absence of benzamidine and PMSF, which indicates that the rate of release of ALPs from tissues without protease inhibitors may be altered by the C-terminal anchor moiety of ALP molecules [23]. However, further addition of several protease inhibitors did not decrease the rate of release of ALPs in the presence of PMSF and benzamidine. Further, the major p*I*s of ALP released from the duodenum in the presence of these protease inhibitors remained unchanged, suggesting that protease(s) in living tissues might be inhibited by these two protease inhibitors.

The rate of release of ALPs from biomembranes might be due to the protein moiety, but the membranous lipid is also involved in the rate of release of ALPs as described above [10, 16, 24]. Both ALPs and acetylcholinesterase are covalently bound through PI on biomembranes [25], and to release ALPs into the medium, PI-specific PLase C is thought to attack the lipid moiety of the enzyme molecules. The other purpose of this work was to assess sugar chains of ALPs in tissues and cultured medium, in order to show the significance not only of protein or lipid moieties but also of sugar moieties with regard to the release of ALPs from biomembranes. We have demonstrated that it is possible to separate ALP isozymes and the enzyme-active forms in human [7] and rats [6] by means of a serial lectin affinity technique established initially by Cummings and Kornfeld

[26]. Fractions isolated by this technique are classified as follows: I, multiantennary complex-type sugar chain; II, biantennary complex type; and III, hybrid type. From the pathway of sugar chain processing, it has been considered that major sugar chains are the high mannose type or hybrid type in the case of active biosynthesizing cells (tissues), and multi- and/or biantennary complex type after the cells have reached maturation.

In this work, we found that the relative activity of fraction III, hybrid-type sugar chain, in the ALP in tissues treated by swainsonine, glucosamine, BDL or partial hepatectomy was significantly increased. This finding is in good accord with the general theory of sugar chain processing. On the other hand, when the duodenum and/or liver were cultured, the ALP having a hybrid-type chain was hardly detected, if at all, in the cultured medium. Even if the duodenal ALP was treated with glucosamine and the relative activity of the hybrid type in the tissue was increased, the ALP released into the medium had a low content of the hybrid type. Consequently, it is suggested strongly that ALPs possessing fractions I and II, complex-type sugar chain, were released in larger amounts than those possessing the hybrid type. ALPs having complex-type chains might be preferentially released. Similar data have been reported for liver ALP from ligated or calmodulin-treated rats [27]. These observations are well supported by evidence that α_1 acute globulin having the complex-type sugar chain is released more than that of the high mannose type [28], and that thyroglobulin possessing more complex moieties than the usual biantennary chains was detected in the culture medium of thyroid cells [29]. Further, a recent report demonstrated that plasma clearance of α_1 acid glycoprotein having complex chains was markedly slower than that with hybrid chains [30], indicating that the sugar chain(s) of glycoprotein in plasma is mainly complex-type chains. Considering these reports, it is confirmed that sugar chain(s) is fractionated into enzyme-active forms without digestion into glycopeptides, and that fractions I and II on the Con A column are clearly ALPs possessing the complex-type sugar chain.

However, when the liver from BDL rats was cultured, ALPs having hybrid-type sugar chains were also released into the medium. This observation is well supported by the finding that solubilized ALPs appeared more in the ligated bile with a detergent-like action [27]. With the liver ALP of the synthesizing-active and/or neonate like cells, ALPs possessing also the hybrid-type sugar chain might be translocated more in their the blood stream. Further, as reported by Young et al. [5], the serum ALP originates from the ileum in the suckling rat, which might be a result of continuous induction by fatty acids in milk [3], and also the induced condition for adult rats. Either a change in the membranous permeability with induction by the content of fatty acids or a shorter half-life of the young cells [31] may alter the rate of release of the ileal ALP having a hybrid-type chain which is less released under normal conditions in adult rats.

From this work, it is possible to suggest that ALPs possessing complex-type sugar chains are mainly released under normal conditions by modification of either protein and/or lipid moieties. On the other hand, the release of ALP into the medium from tissues was inhibited by glucosamine in vitro, indicating an alternative sugar metabolism of glucosamine in vivo, as reported on administration of galactosamine in vivo [32]. Consequently, sugar chains per se may be regulated

to the rate of release of ALPs from tissues. However, how ALPs possessing the complex-type sugar chain are transferred through lining luminal cells to the circulation is an interesting subject for future study.

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