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# THE FUNCTION OF CARBOHYDRATE MOIETY AND ALTERATION OF CARBOHYDRATE COMPOSITION IN HUMAN ALKALINE PHOSPHATASE ISOENZYMES

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## Summary

The relationship between the structure and function of alkaline phosphatase (orthophosphoric-monoester phosphohydrolase (alkaline optimum), EC 3.1.3.1) isoenzymes is under investigation in a number of laboratories. The present study deals with the effects of glycosidase digestion on the alkaline phosphatase isoenzymes. Changes in physicochemical properties, activity, affinity for various lectins and blood group antisera, carbohydrate composition and biological half-life were investigated.

The desialylated hepatic enzyme was shown to be more heat labile and more sensitive to protease digestion in the presence of 0.5% sodium dodecyl sulfate than the native hepatic enzyme.

Helix contents of the native and desialated hepatic enzyme were calculated to be 39.0 and 30.8%, respectively, and apparent molecular weights 175 000 and 167 000, respectively.

Intestinal enzyme preparations treated with α-mannosidase, exo-*N*-acetyl-D-glucosaminidase and endo-*N*-acetyl-D-glucosaminidase-D displayed a decrease in enzyme activity. Among these, the α-mannosidase-treated enzyme activity was the most clearly reduction. The maximum activity of the α-mannosidase-treated intestinal enzyme was observed to change from 40 mM $Mg^{2+}$ to 5—10 mM $Mg^{2+}$.

Abbreviations: The metaplasia enzyme, the enzyme from intestinal metaplasia of the human stomach mucose; treated enzyme 1, desialylated human liver enzyme; treated enzyme 2, desialylated/degalactosylated human liver enzyme; treated enzyme 3, desialylated/degalactosylated/de-*N*-acetyl-D-glucosaminylated human liver enzyme; treated enzyme 4, desialylated/defucosylated human liver enzyme; treated enzyme 5, desialylated human intestinal enzyme; treated enzyme 6, desialylated/degalactosylated human intestinal enzyme; treated enzyme 7, desialylated/degalactosylated/de-*N*-acetyl-D-glucosaminylated human intestinal enzyme; treated enzyme 8, desialylated/defucosylated human intestinal enzyme; SDS, sodium dodecyl sulfate.

Purified intestinal enzyme from adults was found to contain little sialic acid, whereas the fetal intestinal enzyme found to be a sialoglycoprotein. Marked differences were observed with respect to the content of other sugars as well. However, the carbohydrate compositions of adult liver and fetal intestinal enzyme preparations were found to be similar.

Purified alkaline phosphatase from adult human intestine is associated with a sugar chain containing blood group substance. Isoenzymes from human liver, fetal intestine and intestinal metaplasia do not possess the blood group substance.

The half-life of human alkaline phosphatase was found to be 125 h from liver and 7.5 h from intestinal preparations, respectively. Desialylation reduced the half-life of the human hepatic enzyme to 22—25 h but further glycosidase digestion of the carbohydrate moiety restored the half-life to nearly that of the native enzyme.

In the case of the human intestinal enzyme both desialylation plus degalactosylation and desialylation plus defucosylation resulted in a shortening of the half-life to about 1.2 h.

## Introduction

It has been reported by many workers that the alkaline phosphatase (orthophosphoric-monoester phosphohydrolase (alkaline optimum), EC 3.1.3.1) isoenzymes are glycoproteins [1—3]. The authors have further reported that these isoenzymes contain hexosamine, hexose and sialic acid and their respective saccharide content also differs [4,5].

Previous investigations have shown that the functions of the carbohydrate moiety in the enzyme molecules are as follows: (1) providing protection from protease action [5], (2) to act as a negative cooperation factor to enzyme activity [6] and (3) serving as a specific carbohydrate binding protein in the interaction of the enzyme with concanavalin A [5].

In the present study the relationship between the carbohydrate composition and the function(s) of the carbohydrate moiety in the human alkaline phosphatase isoenzymes were investigated.

## Materials and Methods

*Materials.* Alkaline phosphatase preparations were purified from tissue homogenates of human adult and fetal small intestines (proximal region, 23 specimens), human adult liver (13 specimens) and intestinal metaplasia of the human stomach (12 specimens).

The purification procedures were according to the method of Komoda and Sakagishi [5,6] with slight modifications including CM-cellulose chromatography, L-homoarginine/Sepharose 4B affinity chromatography and further concanavalin A/Sepharose 4B affinity chromatography. The specific activities of the preparations were (in $\mu$mol/min per mg): adult liver, 771.3; adult intestine, 513.2, fetal intestine, 909.0; intestinal metaplasia, 345.0.

Sialidase (neuraminate glycohydrolase from *Vibrio cholerae*) was obtained

from B.D.H. Laboratory Chemicals Division, α-D-glucosidase (yeast), β-D-galactosidase (bovine liver), asparagus pea lectin (*Tetragonolobus purpureas*), castor bean lectin (type II), wheat germ lectin, and lithium 3,5-diiodosalycilate were obtained from Sigma Chemical Co. Anti-A, anti-H, anti-Lewis a and anti-Lewis b sera were obtained from Ortho Diagnostic Inc., α-D-mannosidase (Jack bean), exo-*N*-acetyl-D-glucosaminidase (rabbit kidney), and molecular weight estimation standard kits were from Boehringer Mannheim Co. Mixed glycosidase (*Charonia lampas*), α-L-fucosidase (*Turbo cornutus*), α-D-galactosidase (*T. cornutus*), exo-*N*-acetyl-D-galactosaminidase (*Ch. lampas*) and concanavalin A (thrice crystallized) were from Miles Laboratories Inc., pronase-P from Kaken Chemicals Co. (Tokyo) and endo-*N*-acetyl-D-glucosaminidase-H (*Streptomyces griseus*), endo-*N*-acetyl-D-glucosaminidase-D (*Diplococcus pneumonia*) from Seikagaku Ind. Co. (Tokyo). All other chemicals were analytical grade from Wako Pure Chemical Co. (Osaka).

*Purification of the commercial glycosidases.* Preparation of the protease-free sialidase was carried out according to the method of Winkelhake and Nicolson [7], that of the highly purified β-galactosidase was carried out by the method of Norden and O'Brien [8], and that of the exo-*N*-acetyl-D-glucosaminidase was carried out by the method of Verpoorte [9].

*Enzyme assays and protein determinations.* The reaction mixture contained 2 mmol disodium *p*-nitrophenyl phosphate in the presence of 1 mM $CaCl_2$/50 mM carbonate/bicarbonate (pH 10.0) at 30° C. The enzyme activity was determined according to the procedures described earlier [6].

Protein concentration was estimated according to Hartree [10] using bovine serum albumin as a standard.

*Hydrolysis of alkaline phosphatase by various glycosidases or pronase.* For the hydrolyss of alkaline phosphatase by sialidase, α-galactosidase, β-galactosidase, α-glucosidase, α-fucosidase, α-mannosidase, exo-*N*-acetyl-D-glucosaminidase, exo-*N*-acetyl-D-galactosaminidase, endo-*N*-acetyl-D-glucosaminidase-H or endo-*N*-acetyl-D-glucosaminidase-D, a solution containing 0.1 unit/ml of respective glycosidase was added to an equal volume of the enzyme in 1 mM $CaCl_2$/20 mM acetate buffer (pH 6.0). A mixture of the enzyme and the respective glycosidase was incubated for 24 h at 37° C.

The hydrolysis of alkaline phosphatase by pronase-P was carried out according to the method of Komoda and Sakagishi [5,11].

*Carbohydrate analysis.* Total carbohydrate concentrations were determined by the method of Krystall and Graham [12]. Amino sugars were detected and determined on the amino acid analyzer after partial hydrolysis of the enzyme preparation in 4 M HCl in evacuated tubes at 110° C for 4—8 h. Because of the low galactosamine content in the complex, a known amount of galactosamine was added to the sample before analysis. Quantitative estimation of neutral sugar by gas liquid chromatography was carried out according to the trifluoroacetylation method with *N*-methylbistrifluoroacetoamide [4,5]. Determination of sialic acid was performed according to the methods of Hess and Rolde [13] or of Komoda and Sakagishi [6].

*Deglycosylated alkaline phosphatases.* The respective deglycosylated enzymes were purified by chromatography on DEAE-cellulose columns equilibrated with 20 mM Tris · HCl (pH 8.). The resulting main fraction was con-

TABLE I

THE SPECIFIC ACTIVITIES OF HUMAN ALKALINE PHOSPHATASE TREATED WITH DE-GLYCOSYLATING ENZYMES

| Source of enzyme | Treatment | Specific activity (μmol/min per mg) |
|---|---|---|
| Liver | sialic acid | 901.3 |
| | sialic acid/galactose | 875.2 |
| | sialic acid/galactose/*N*-acetylglucosamine | 819.7 |
| | sialic acid/fucose | 828.6 |
| Intestine | sialic acid | 528.1 |
| | sialic acid/galactose | 541.6 |
| | sialic acid/galactose/*N*-acetylglucosamine | 478.3 |
| | sialic acid/fucose | 509.7 |

centrated in a collodion bag (Sartorius Membrane Filter, SM 13 200) and passed through a Sephadex G-150 column equilibrated with50 mM Tris · HCl (pH 8.4). The central portion of the resulting peak was dialyzed exhaustively against 20 mM Tris · HCl (pH 8.4). The deglycosylated enzyme preparations had specific activities, as shown in Table I.

*Cellogel electrophoresis and partial specific volume determinations.* Procedures of Cellogel membrane electrophoresis and specific staining for the alkaline phosphatase were as described earlier [6].

The partial specific volume of the native enzyme was measured at 20° C with a digital precision densitometer (DMA 02C, Anton Parr K.G., Graz, Austria). A large series of density measurements was carried out over a concentration range of 0.5—2%. Partial specific volume calculations were based on the value of $\bar{v}$ for bovine serum albumin obtained by using the same technique.

*Sedimentation velocity determinations.* Sedimentation velocity experiments were carried out in a Beckman Spinco Model E analytical ultracentrifuge using a standard cell in an An-D rotor. The rotor speed was 59 640 rev./min and the temperature was 20° C. Protein samples were dialyzed against and diluted with Tris/borate buffer (pH 6.0).

*Molecular weight determinations with gel filtration.* Sephadex G-150 filtration experiments were carried out according to the method of Fosset et al. [3].

*Heat stability measurements.* The reaction mixture was incubated in the cuvette of a spectrophotometer (UV-200, from Shimazu Instrument Co., Kyoto) equipped with a thermospacer connected to a constant temperature water bath (CTE-220 and CTR-220, from Komatsu-Yamato Science Co. Ltd.). The enzyme activity was calculated from the increase in absorbance at 405 nm for 3—10 min after addition of substrate. For heat treatment preparations were incubated for 5 min and assayed under standard conditions.

*α-Helix contents.* Circular dichroism spectra were measured at room temperature with a Jasco recording spectropolarimeter, Model J-20, with a circular dichroism attachment which had been calibrated with d-10-camphorsulfonic acid [14,15]. The mean residue ellipticity, $[\theta]$, was obtained from the equation,

$$[\theta] = \frac{100 \cdot \theta}{l \cdot c}$$

where $\theta$ is the observed ellipticity in degrees, $c$ the residue molar concentration of alkaline phosphatase, and $l$ the path length of the cell in centimeters.

*Blood group A, H, Lewis a and Lewis b activities on the enzyme.* ABH blood grouping was performed by a hapten inhibition test [16,17] using a microtitration system. Activities for Lewis a and Lewes b specificities were further tested by precipitin reaction with anti-Lewis a or anti-Lewis b goat antisera of Marcus and Grollman [18] on agar diffusion plates.

Preparation of Sepharose-coupled anti-A and anti-H antisera was carried out by the methods of Cuatrecasas and Anfinzen [19] and Kelly and Alpers [17] with slight modifications. The anti-A or anti-H antisera/Sepharose gel (0.8 × 10 cm) was equilibrated with 20 mM phosphate buffer (pH 6.5) and the alkaline phosphatases then reacted with the antisera/Sepharose gel. Elution was performed with 20 mM phosphate buffer (pH 6.5).

*Turnover studies of the enzymes.* Turnover studies were performed using 20 New Zealand White rabbits with an average body weight of 3.0 kg. All rabbits received standardized diets and drinking water containing 0.005% NaI (w/v). The non-treated and deglycosylated enzyme preparations were labelled with $Na^{125}I$ (New England Nuclear Co.) according to the method of Greenwood et al. [20], and the iodonated enzyme was isolated by DEAE-Sephadex chromatography. Rabbits were injected simultaneously with 0.05 mg labelled deglycosylated enzyme or with 0.05 mg labelled non-treated human alkaline phosphatase preparations. The amount of labelled enzyme in the blood stream of the rabbits was monitored for a total period of 150 h after injection. Blood samples were precipitated with 1.0 ml cold 4% phosphotungstic acid in 2 M HCl. The precipitate was centrifuged, the resulting pellet dissolved in 0.5 ml protosol (New England Nuclear Co.) and added to 10 ml toluene/Triton X-100. The solution was neutralized by the addition of 0.1 ml glacial acetic acid and the radioactivity determined in a Beckman LS-150 liquid scintillation counter.

## Results

*Alteration of electrophoresis patterns by glycosidase treatment.* Of the various exo-glycosidases, only sialidase treatment altered the rate of migration of alkaline phosphatase from human liver during electropherogram, whereas the rate of migration of the treated enzyme 5 was not changed (data not shown).

*Double reciprocal plots of deglycosylated alkaline phosphatase.* As shown in Table II, sialidase treatment altered the $K_m$ value of the hepatic enzyme. Similar results have been reported previously [6]. But, in the pH dependence of the log $K_m$ values there was a marked difference between the native intestinal enzyme and native or sialidase-treated enzymes (unpublished data).

*Thermal and pronase-P inactivation of the native and desialylated alkaline phosphatase.* Table II also shows results of heat treatment and protease digestion of the enzyme preparations. Thermal inactivation curves in the range of 40—56° C were identical for the native and the desialylated enzymes. However, while 0.5% sodium dodecyl sulfate (SDS) at 30° C produced no inhibition of the native enzyme, the treated enzyme 1 showed reduced activity at 56° C in the presence of 0.5% SDS.

Incubation with pronase-P for 60 min resulted in a 52—55% loss in the activ-

TABLE II

EFFECTS OF HEAT AND PRONASE TREATMENTS ON THE NON-TREATED AND THE DESIALYLATED HUMAN ALKALINE PHOSPHATASE ACTIVITIES

The enzyme concentrations were used with 0.13 μg human liver (blood type A) and 0.25 μg human intestine (blood type A), respectively. The enzyme preparations were obtained from human adult tissues. All other conditions used were the same as described under Materials and Methods.

| | Non-treated alkaline phosphatase | | Desialylated alkaline phosphatase | |
|---|---|---|---|---|
| | Liver | Intestine | Liver | Intestine |
| $K_m$ for *p*-nitrophenyl phosphate (μM) | 67 | 92 | 83 | 92 |
| Optimum pH | 10.2 | 9.8 | 10.3 | 9.9 |
| Percent of remaining activity | | | | |
| at 56°C for 10 min | 61 | 68 | 58 | 67 |
| at 56°C for 10 min and after that added with 0.5% SDS | 50 | 67 | 32 | 65 |
| digested with pronase-P at 37°C for 60 min | 45 | 48 | 40 | 46 |
| digested with pronase-P at 37°C for 60 min and after that added with 0.5% SDS | 29 | 30 | 18 | 28 |

ity of the native alkaline phosphatases, and an even greater loss of 54—60% of the activity of the sialidase-treated enzymes. The difference in susceptibility is shown more clearly the treated enzyme 1 in the presence of 0.5% SDS.

*Conformation of the native and desialylated enzymes.* The results of circular dichroism spectra indicate that the human alkaline phosphatases have about 65% random structure. The helix contents of the enzyme have about 40% from liver and about 30% from intestine, respectively. The α-helix content of the treated enzyme 1 was reduced from 39.0% (non-treated hepatic enzyme) to 30.8%. It is possible that the sialidase treatment causes a conformational change in the hepatic alkaline phosphatase structure.

*Molecular properties of the native and desialylated enzymes.* The apparent partial specific volume of the intestinal and the hepatic enzymes are quite different being 0.746 and 0.730, respectively, but there is no change in value between the native and the desialylated forms. Sedimentation coefficients at pH 6.0 and ionic strength 0.35 were found to be identical for the native and desialylated forms, as were the values of $s^0_{20,w}$ (about 6.7—6.8 S). There was a slight difference in apparent molecular weight as determined by gel filtration, values obtained being 175 000 daltons for the native hepatic enzyme and 167 000 daltons for the treated enzyme 1.

*Effects of various glycosidases on the activities of the two alkaline phosphatases.* Treatment with α-mannosidase and exo-*N*-acetyl-D-glucosaminidase or endo-*N*-acetyl-D-glucosaminidase-D clearly decreased the activity of the intestinal enzymes but showed little if any effect on the hepatic enzyme (Table III). Most marked reduction in activity occurred with α-mannosidase treatment of the intestinal enzyme, activity being reduced to 45—55% of that of the native enzyme. pH dependence and $K_m$ values of the intestinal enzyme were not altered by any of above treatments.

The enhancement of human intestinal alkaline phosphatase activity by $Mg^{2+}$ is shown in Fig. 1. A marked difference was observed between the maximum

TABLE III

EFFECTS OF VARIOUS GLYCOSIDASE TREATMENTS ON THE LIVER AND INTESTINAL ALKALINE PHOSPHATASE ACTIVITIES

The enzyme assay conditions were carried out with 2 mmol disodium *p*-nitrophenyl phosphate (magnesium free). Alkaline phosphatases used were obtained from blood group A type human liver (0.78 μg) and human intestine (0.85 μg). All of the enzymes were from adult tissues.

| Treatment of various glycosidases | Liver | Intestine |
|---|---|---|
| Non-treated | 0.602 | 0.438 |
| α-Mannosidase | 0.621 | 0.248 |
| Exo-*N*-acetyl-D-glucosaminidase | 0.627 | 0.302 |
| Endo-*N*-acetyl-D-glucosaminidase-H | 0.581 | 0.434 |
| Endo-*N*-acetyl-D-glucosaminidase-D | 0.567 | 0.299 |

activation of the native and the α-mannosidase-treated enzyme, actual values obtained being 40 mM $MgCl_2$ for the native and 5—10 mM $MgCl_2$ for the treated intestinal enzyme. This difference in optimum magnesium concentration could be demonstrated more clearly in the presence of Tris · HCl buffer than with carbonate/bicarbonate buffer. Exo-*N*-acetyl-D-glucosaminidase or endo-*N*-acetyl-D-glucosaminidase-D treatment of the intestinal enzyme did not alter the optimal magnesium concentration for enhancement.

Electrophoretic studies pointed to a difference in affinity for concanavalin A between the various enzyme preparations. The native [5], the sialidase-treated enzyme and the endo-*N*-acetyl-D-glucosaminidase-H-treated enzyme were shown to have a clear affinity for concanavalin A, whereas alkaline phosphatase treated with endo-*N*-acetyl-D-glucosaminidase-D or α-mannosidase had very little affinity. Similar differences were observed with respect to affinity for

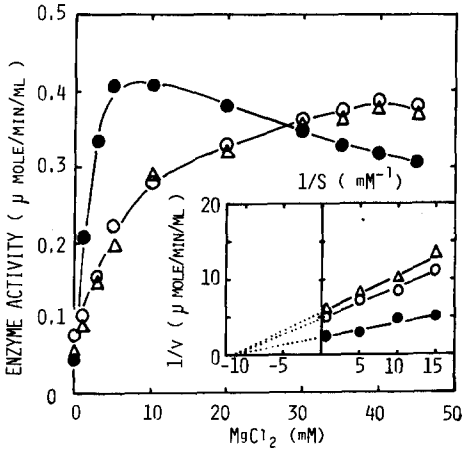


Fig. 1. The effect of $MgCl_2$ on the activity of intestinal alkaline phosphatase. Enzyme assays were performed as described under Materials and Methods. Results are shown for 0.16 μg non-treated intestinal enzyme (○), 0.14 μg intestinal enzyme treated with endo-*N*-acetylglucosaminidase-D (△) and 0.15 μg intestinal enzyme treated with α-mannosidase (●). The inset in Fig. 1 shows Lineweaver-Burk plots of reciprocal substrate concentration. Assay conditions for phosphatase activity are described in Methods. The concentration of $Mg^{2+}$ used was 5 mM.

TABLE IV

QUANTITATIVE ESTIMATION OF THE CARBOHYDRATE COMPOSITION OF VARIOUS HUMAN ALKALINE PHSOPHATASE PREPARATIONS

Calculation were based on a molecular weight of 157 000. All the enzymes were obtained from subjects with type A and Lewis b blood groups. Sugar determinations were performed as described under Materials and Methods, and a minimum of three samples were used for each determination. Specific activities of the adult and fetal intestinal enzymes were determined after additional purification by 4.5% polyacrylamide gel disc electrophoresis [5,6]. Alkaline phosphatase activity was eluted from the gels with 20 mM Tris · HCl, pH 7.5, containing 0.01 mM $ZnCl_2$. ALP, alkaline phosphatase; AL, adult liver; AI, adult intestine; FI, fetal intestine; IMS, intestinal metaplasia of the adult stomach mucosa. The exhibited specific activities of enzymes used were 1020 μmol/min per mg (FI-ALP) and 1140 μmol/min per mg (AI-ALP).

| Carbohydrates | Mol of carbohydrate per mol of enzyme | | | |
|---|---|---|---|---|
| | AL—ALP | AI-ALP | FI-ALP | IMS-ALP |
| Galactose | 10.8—12.4 | 11.0—13.7 | 9.0—11.7 | 6.2— 8.4 |
| Glucose | 2.8— 3.4 | 3.3— 4.0 | 2.6— 3.3 | 5.3— 6.0 |
| Mannose | 5.7— 6.1 | 6.2— 8.3 | 6.4— 8.0 | 7.1—13.0 |
| Fucose | 4.5— 4.8 | 10.2—10.8 | 8.3— 9.1 | 4.1— 7.2 |
| Sialic acid | 14.2—22.1 | trace | 10.5—12.0 | 2.3— 6.8 |
| Galactosamine | 5.1— 6.3 | 8.5— 9.3 | 6.2— 7.0 | 4.8— 6.6 |
| Glucosamine | 27.8—31.9 | 15.3—18.8 | 17.4—20.4 | 15.1—22.5 |

other lectins. While the untreated fetal and hepatic enzyme coupled to wheat germ lectin, the treated enzyme 1, the fetal intestine enzyme and the intestinal metaplasia enzyme had little affinity. The non-treated, the sialidase-treated and the galactosidase-treated enzymes had an affinity for castor bean lectin whereas the treated enzyme 2 had little affinity. Studies were also performed to determine affinity for asparagus pea lectin, a fucose-binding protein. Affinity was demonstrated in the case of the treated enzyme 4 and to a lesser extent with the treated enzyme 4. Moreover, the adult intestinal enzyme showed a greater affinity than that from fetal or intestinal metaplasia sources (unpublished data).

*Differences in carbohydrate composition among adult intestinal enzyme, fetal intestine enzyme and enzyme from intestinal metaplasia.* The carbohydrate compositions of the three human intestinal alkaline phosphatases are shown in Table IV. While the total carbohydrate content of the various enzymes was almost identical, fetal enzyme and intestinal metaplasia enzyme clearly contained sialic acid, and more glucosamine and had a lower content of fucose, galactose and galactosamine than the adult intestinal enzyme. This difference in the carbohydrate composition further supports observations of lower affinities of the fetal and the intestinal metaplasia enzyme for asparagus pea lectin as a fucose-binding agent.

*Association of blood group reactivity with the isolated enzymes.* Purified enzyme was incubated with the blood group antibodies (ABH or Lewis) then, either assayed for phosphatase activity or subjected to Cellogel electrophoresis. It has been demonstrated that the addition of antibody does not effect the enzyme activities and electrophoretic mobility.

When the affinity between blood group type A adult intestinal enzyme and

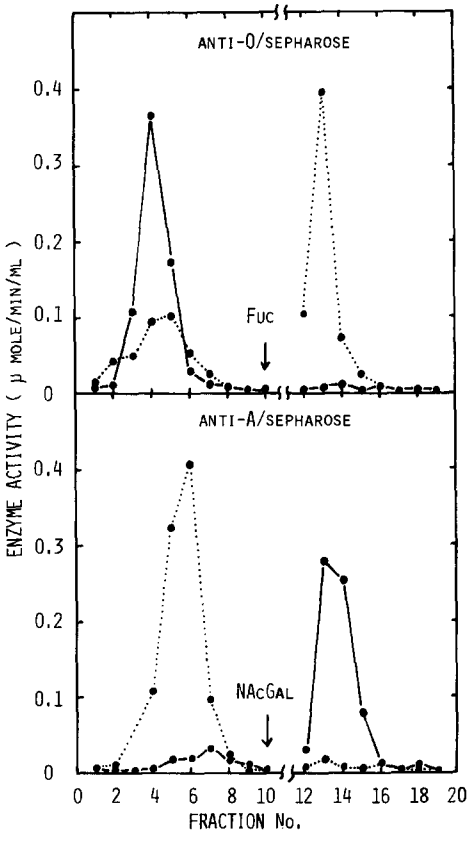


Fig. 2. Antibody/Sepharose affinity chromatography of human alkaline phosphatase. Samples of 3.4 μg alkaline phosphatase from blood group A type or O type patients prepared as described under Materials and Methods were added to an affinity column of 0.8 × 10 cm size. $V_0$ of the column was 2.8 ml and the fraction size was 1.0 ml. The column was washed with buffer of 14 times $V_0$. Enzyme activities were eluted with 0.6 M *N*-acetylgalactosamine (NAcGal) or 1 M L-fructose (Fuc) in 20 mM phosphate buffer, pH 6.5, in the presence of 0.01 mM $ZnCl_2$. Top: ———, enzyme from fetal intestine (blood type A); · · · · · ·, enzyme from adult liver (blood type A). Bottom: ———, enzyme from adult intestine (blood type A); · · · · · ·, enzyme from adult liver (blood type A).

anti-O antisera/Sepharose gel was tested virtually all of the activity passed through the column while almost all of the activity was retained on a column prepared with anti-A antisera (Fig. 2). Moreover, virtually all the type A and type O intestinal enzyme activity could be eluted from the column using 0.6 M *N*-acetyl-D-glucosamine or L-fucose. Little enzyme was retained by a column of anti-A antibody/Sepharose in the case of type A human hepatic, fetal intestine or intestinal metaplasia enzymes, whereas these were retained to a considerable extent by column prepared with anti-O antibody.

The above results were not surprising in view of the close relationship between appearance of alkaline phosphatase in the blood stream to the ABH blood groups [21,22] and the secretory and non-secretory factors of the Lewis blood groups [23,24]. The enzyme from type A fetal intestine and intestinal metaplasia had a lower affinity for anti-A antisera/Sepharose gel than the adult intestinal enzyme. This observation was confirmed by the results of affinity

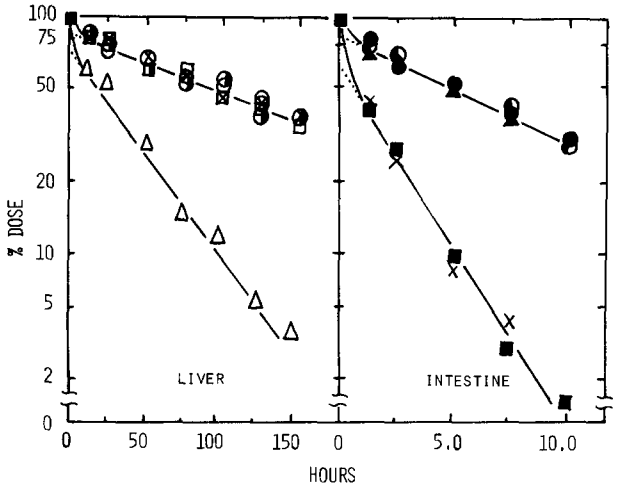


Fig. 3. Protein-bound radioactivities in the plasma of rabbits following the injection of $^{125}$I-labelled native enzyme and $^{125}$I-labelled deglycosylated alkaline phosphatase from patients with blood groups O type and Lewis b. Inset (A): ○, the native liver enzyme; △, treated enzyme 1; □, treated enzyme 2; ◑, treated enzyme 3; ⊗, treated enzyme 4. Inset (B): ●, the native intestinal enzyme; ▲, treated enzyme 5; ■, treated enzyme 6; ◐, treated enzyme 7; X, treated enzyme 8. All of the above alkaline phosphatases were from adult human tissues.

studies with asparagus pea lectin and the differences in carbohydrate composition reported above.

*Turnover rates of the native and deglycosylated human alkaline phosphatase isoenzymes in the rabbit.* Intravascular distributions of $^{125}$I-labelled native human enzyme (40—60 μCi/mg protein) and the $^{125}$I-labelled deglycosylated human enzyme (40—60 μCi/mg protein) are shown in Fig. 3.

The biological half-life of the native human liver alkaline phosphatase and the intestinal enzyme was found to be 125 and 7.5 h, respectively. Biological half-life was clearly shown to decrease as a result of desialylation but was restored to that of the native hepatic enzyme when further glycosidase digestions was performed. In the case of the intestinal enzyme, the reduction in half-life of the treated enzyme 6 and the treated enzyme 8 was greater than that of the treated enzymes 5 and 7. Moreover, there was no difference in turnover rates between the native and the deglycosylated enzymes. The half-life of native adult intestinal enzyme and that of the enzyme from intestinal metaplasia were almost identical. We obtained identical results when the half-life of $^{125}$I-labelled enzyme from rabbit tissues was examined.

## Discussion

Experiments on sialidase treatment of alkaline phosphatase isoenzymes indicate that a possible role of the sialic acid residue may be to protect the active conformation through the carbohydrate moiety which when present increase stability of the protein moiety to heating and pronase digestion and maintains the three-dimentional structure of the protein. The fact that sialidase treatment causes conformational changes has already been demonstrated in the case of

human prostate acid phosphatase [25]. It has been reported that sialidase treatment does not significantly affect other properties of the human liver alkaline phosphatase, e.g. inactivation by addition of 10 mM L-homoarginine, or antigenicity [6].

Treatment with α-mannosidase caused a shift in the optimum magnesium concentration of the intestinal enzyme. The reason for this is not yet clear. A possible reasons may be a change in the affinity of the enzyme as a result of the presence of mannose or a change in conformation.

It has been previously reported that *N*-acetyl-D-glucosaminidase-H is specifically hydrolyzed with the sugar chain containing a so-called "mannose-rich" saccharide [26] and endo-*N*-acetyl-D-glucosaminidase-D is specifically hydrolyzed with the sugar chain containing "sialic acid-galactose-*N*-acetylglucosamine [27]. Present affinity studies with various lectins suggest that the sugar chain of alkaline phosphatase is similar to the sugar chain of "sialic acid-galactose-*N*-acetylglucosamine", and galactose sits next to the terminal sialic acid as is known to be the case with secretory glycoproteins.

Differences observed in carbohydrate composition between the adult and fetal enzymes may be due to *Escherichia coli* exhibiting sialidase activity in adult intestinal mucosa but not in the fetal intestine which is thought to be free of such bacteria [28]. Furthermore, it is interesting to consider the similarities in carbohydrate composition between the enzyme from fetal intestine and adult liver and the differences in carbohydrate composition between enzyme from fetal intestine and intestinal metaplasia enzyme.

As reported in the case of human intestinal sucrase [17], purified adult intestinal alkaline phosphatase is also associated with blood group components. Previous findings have also shown that the blood group component of tissue from intestinal metaplasia is considerably lower than that of normal adult intestinal tissue [29].

Turnover studies have demonstrated a marked difference in half-life between enzymes from different organ sources, the half-life of intestinal enzyme being much lower than that of the hepatic enzyme. Similar results have been reported by Posen [30]. The mechanism by which the enzyme disappears from the blood stream and the relationship between deglycosylation and changes in half-life are not yet clear. However, it is possible that the degradation of the enzyme is carried on by the reticuloendothelial route the same way as in the case of secretory glycoproteins [31—33].

From the above we may conclude that the terminal carbohydrate moiety of the alkaline phosphatase molecule is closely related to the recognized secretive and metabolic properties, and to the half-life rate of the enzyme.

Investigations of the galactose specific, defucosylated glycoenzyme and the de-*N*-acetyl-D-glucosaminylated glycoenzyme specific receptor sites [34] are now in progress.

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