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SUGAR-CHAIN HETEROGENEITY OF HUMAN ALKALINE PHOSPHATASES: DIFFERENCES BETWEEN NORMAL AND TUMOUR-ASSOCIATED ISOZYMES

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SUMMARY

The sugar-chain heterogeneity of alkaline phosphatases (ALPs) from various human organs was investigated by using the serial lectin affinity technique. This technique revealed a possible structure of the sugar chain(s) of ALP isozymes and clarified a difference in affinity on the lectin column not only among three genetically different isozymes (liver/bone/kidney, intestinal and placental types) but also among liver, bone, and kidney ALPs. Lectin-binding profiles of ALPs in these human organs closely resembled those in the corresponding organs of the rat, as reported previously, suggesting that heterogeneities in sugar chains of ALPs have a specificity for the respective organs rather than being species-specific. Lectin-binding profiles of tumour-produced placental and liver ALPs were significantly different from those of ALPs in the respective normal organs. However, the two altered ALPs exhibited similar lectin-binding affinities. Isoelectric focusing analysis showed essentially no difference in protein charge between the normal and tumour-produced ALPs. Moreover, tumour-produced ALPs had the same N-terminal amino acid sequence and peptide mapping as normal ALPs. From these results, it is possible to suggest that organ-specific sugar chains in ALP isozymes are changed into those peculiar to tumours in association with malignant transformation.

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INTRODUCTION

Human alkaline phosphatases (ALPs, EC 3.1.3.1) possess at least three isozymes: liver/bone/kidney (tissue-unspecific), intestinal and placental types, which are genetically different from each other. In contrast, the tumour-produced placental ALP is well expressed as an ectopic product in different human tumours. This isozyme is often detected in serum, in association with a tumour originating in an organ such as the lung (Regan or Nagao isozyme), and closely resembles human term placental ALP by enzymological and immunological examination [1,2]. These tumour-produced placental ALPs have been further characterized by heat stability, inhibition by L-leucine and electrophoretic mobility [3].

With the glycoprotein used as a tumour marker, structural studies of sugar chains have been performed by using the serial lectin affinity technique, which was established by Cummings and Kornfeld [4]. Yamashita et al. [5] have reported that the structure of the sugar chain in γ-glutamyltranspeptidase (γGTP, EC 2.3.2.2) associated with primary hepatoma is different from that of the enzyme in normal liver. α-Fetoprotein was also studied with regard to differences in sugar structure between normal and tumour organs [6]. Tumour-produced glycoproteins seem to possess bisecting N-acetylglucosamine (GlcNAc) or a more complex structure in the sugar chains because of the expression or the deficiency of some glycosyltransferases in association with tumours [7]. It is therefore possible that the sugar-chain structure of ALPs may also be changed by malignant transformation. Although we previously reported the carbohydrate composition of human liver and intestinal ALPs [8,9] and the physiological role of the sugar moieties in ALPs [10], no structural studies of the sugar chains in human ALPs have been conducted as yet. It is important for the understanding of the malignant change in tumours to investigate not only the protein moiety but also the structures of the sugar chains in the altered ALP forms.

We previously developed the serial lectin affinity technique for the separation of rat ALP isozymes as the enzyme's active forms, and demonstrated that it is possible to postulate the sugar-chain type of intact ALP molecules without using glycopeptides isolated from the isozymes [11]. In the present study, we investigated the structures of the sugar chains in ALPs from various human organs by means of this lectin affinity technique. This paper describes mainly the organ-specific heterogeneity of sugar chains in ALPs and compares the sugar chains of normal and tumour-produced ALPs.

EXPERIMENTAL

Enzyme preparations and enzyme assay

Human liver, kidney, bone (costal cartilage), duodenum and term placenta were used as sources of the enzyme. Each sample was homogenized in 5 vols. of 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, at 4°C for 1 min in a Waring blender. The homogenates were then centrifuged at 15 000 *g* for 15 min, and the resulting supernatant was retained as the enzyme source. The crude

ALP samples were then partially purified as described previously [11]. In order to obtain the whole enzyme form, including the minor enzyme forms, the extracts were first treated with 20% *n*-butanol at 4°C for 1 h to remove the lipid residue in the crude enzyme, concentrated by precipitation with 60% acetone and then gel-chromatographed with Sephacryl S-200 (Pharmacia, Uppsala, Sweden). By the above purification steps, the following enzyme-specific activities (in μmol/min per mg of protein) in each sample were obtained: liver, 1.6; kidney, 0.4; bone, 0.01; duodenum, 1.0; and term placenta, 10.9. Hepatoma-produced ALP and malignant omentum-produced placental ALP were also partially purified from liver and omentum, respectively, as well as normal organs, and the enzyme-specific activities of two ALPs were determined as 1.8 and 8.7, respectively. These samples were then used for the lectin affinity chromatography.

All the samples were assayed for ALP activity, which was measured by the release of *p*-nitrophenol from disodium *p*-nitrophenylphosphate at 405 nm. The assay was performed with 50 m*M* carbonate—bicarbonate buffer, pH 10.0, containing 5 m*M* magnesium chloride, at 37°C as described previously [12]. Protein concentrations were determined according to Lowry et al. [13] with bovine serum albumin used as a standard.

Lectin affinity chromatography

The lectins used were concanavalin A (*Canavalia ensiformis*, Con A) purchased from Pharmacia; pea lectin (*Pisum sativum*, PSA) which recognizes the internal fucose residue; wheat germ agglutinin (*Triticum vulgaris*, WGA) which interacts strongly with GlcNAc and less with sialic acids; and phytohemagglutinin E (*Phaseolus vulgaris*, PHA-E) which has major binding specificity for $\beta(1\rightarrow4)$GlcNAc [11], purchased from E.Y. Labs. (U.S.A.). A column (10×0.5 cm I.D.) of Con A—Sepharose or PSA— or WGA—agarose, 2 ml, was equilibrated with 10 m*M* of Tris—HCl-buffered saline (TBS), pH 8.0, supplemented with 1 m*M* each of calcium chloride, magnesium chloride and manganese chloride, and 10 μ*M* zinc chloride, and a column of PHA-E—agarose was equilibrated with phosphate-buffered saline (PBS), pH 7.5, containing the same metal ions as TBS. Lectin affinity chromatography was performed as described previously [11]. Briefly, an enzyme preparation with a specific activity of 0.2 μmol/min was applied to the Con A column, and allowed to stand at room temperature for 3 h. Three fractions were then obtained by using two different concentrations (0.01 and 0.5 *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). These fractions were further applied to the PHA-E, the PSA and the WGA columns, respectively. The unbound and the bound fractions, designated as A and B, were separated on the respective columns by using 0.1 *M* GlcNAc, 0.2 *M* αMM, or 0.1 *M* GlcNAc, respectively, as an elution buffer.

WGA affinity electrophoresis for ALPs

Electrophoresis was carried out on a cellulose acetate plate, Titan III (Helena, U.S.A.), immersed for 30 min in a 50 μg/ml solution of WGA in Tris—barbital buffer (pH 8.0, $\mu=0.06$). ALP preparations were loaded for 30 min at 180 V and

then stained with a solution of 5 m*M* 5-bromo-3-indolylphosphate *p*-toluidine salt (BIP) (Wako, Japan) in 1.0 *M* amediol—HCl bufffer, pH 10.2, containing 1 m*M* magnesium chloride and 10 μM zinc chloride at 37°C. The ALP preparation, which was digested with 10 μg/U ALP of subtilisin (Sigma, St. Louis, MO, U.S.A.) [14] for 1 h or 0.1 U/ml endo-N-acetylglucosaminidase F (Boehringer Mannheim Yamanouchi, Japan) [15] for 3 h at 30°C, was also subjected to WGA affinity electrophoresis.

Analysis of protein moieties in ALPs

The ALP preparations to be used for isoelectric focusing were treated for 3 h at 25°C with phosphatidylinositol-specific phospholipase C (PI-specific PLase C, 1 mU/ml) [16], kindly supplied by Dr. H. Ikezawa of Nagoya City University (Japan), in order to remove PI residues in the enzyme preparation, which are involved in the attachment of ALPs to biomembranes [17]. The ALP preparations were further digested with 100 mU/ml neuraminidase (Nakarai, Japan), to eliminate the charge of the sialic acids [18]. The resulting ALP preparations with a specific activity of 2–8 nmol/min per tube, supplemented with 25% glycerol—0.1% sodium dodecyl sulphate (SDS)—0.005% bromophenol blue, were 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 a preloading for 20 min at 50 V, the preparations in the gels were focused for 30 min at 300 V. The gels were stained for ALP activity with BIP at 37°C.

Analysis of N-terminal amino acid sequence of ALPs was carried out as described earlier [19]. For this analysis, the partially purified ALP preparations were further purified by polyclonal antibody immunoaffinity chromatography, as described by Vockley and Harris [20]. Enzyme-specific activities of tumour-produced liver and placental ALPs were 720 and 850 μmol/min per mg of protein, respectively.

The peptide mapping for the primary structure of liver and hepatoma ALPs was carried out by the method of Cleveland et al. [21], with minor modifications [22]. The preparations treated as those to be used for isoelectric focusing, were labelled at the active site with ^{32}P (carrier free, 10 mCi/ml, NEN, Boston, MA, U.S.A.) at 0°C as reported by Carlson et al. [23]. The resulting ^{32}P-labelled preparations were partially digested for 30 min with *Staphylococcus aureus* V8 protease (Sigma) in the presence of 0.1% SDS. Thereafter, 0.5% β-mercaptoethanol was added and heated at 100°C for 3 min. The resulting ^{32}P-labelled, active-site-containing ALP polypeptide fragments were then run on a 0.1% SDS – 10% polyacrylamide gel. Resulting gels were soaked in EN^{3}HANCE (NEN) for 1 h, dried, and visualized by fluorography [22].

RESULTS

Lectin affinity chromatography of ALPs from various human organs

The Con A elution profiles of the ALPs were obviously different for each organ when two different concentrations of αMM as competitive sugars were used (Fig. 1). In particular, liver, bone and kidney ALPs as the tissue-unspecific type

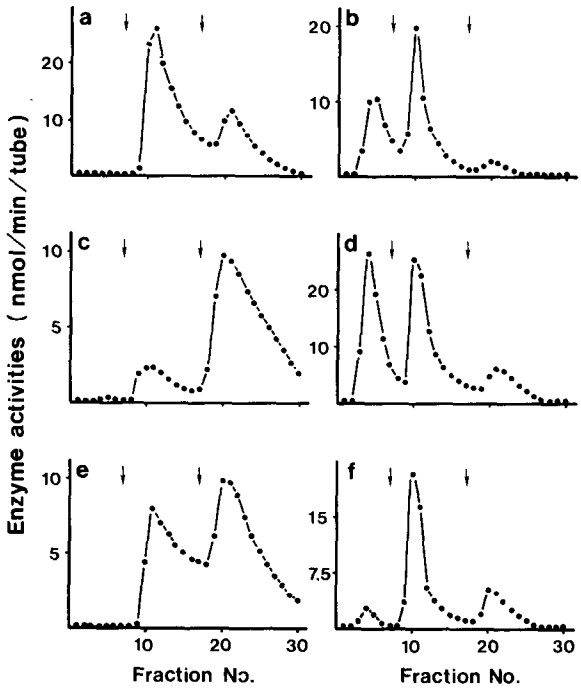

Fig. 1. Isolation of multiple forms of human ALP isozymes by Con A affinity chromatography. Human ALPs in (a) liver, (b) kidney, (c) bone, (d) duodenum, (e) term placental and (f) rat placental ALP were applied to a Con A column. After washing, two fractions were eluted with 0.01 and 0.5 *M* αMM as described in the text (indicated by the arrows).

enzymes were distinguishable among the ALPs in these three organs. The fraction III content of the tissue-unspecific type enzymes was in the order kidney < liver < bone. On the other hand, the kidney ALP as well as the duodenal enzyme was rich in fraction I. Furthermore, elution profiles of liver, bone, kidney and duodenal ALPs closely resembled those of rat ALPs from the respective organs reported previously [11]. However, those of human term placental ALP were different from those of rat placental ALP (Fig. 1e and f), which may be due to the fact that rat placental ALP is classified enzymologically as a tissue-unspecific type enzyme [24]. Recoveries from the Con A column of the respective isozymes in the organs were 90–100%.

In order to evaluate further the lectin-binding affinity of each isozyme, fractions I, II and III obtained on the Con A column were further applied on a PHA-E, the PSA or WGA column, respectively, to separate them into the unbound (A) and the bound (B) fraction. Fraction I in the organs tested bound virtually 100% to the PHA-E column. Accordingly, five fractions were then obtained by the serial lectin affinity technique: I, the fraction passing through the Con A column and binding to the PHA-E column; IIA, the fraction bound weakly to Con A and passing through the PSA column; IIB, the fraction bound weakly to Con A and bound clearly to PSA; IIIA, the fraction bound strongly to Con A and passing through WGA; and IIIB, the fraction bound strongly to Con A and to

TABLE I

RELATIVE AMOUNTS OF THE FIVE FRACTIONS OF ALPs IN VARIOUS ORGANS

The fractions were obtained by the serial lectin affinity technique as described in the text. The values represent the enzyme activity of the five fractions expressed as the percentage of the total activity.

Fraction	Amount of each fraction in ALP activity (%)				
	Liver	Bone	Kidney	Duodenum	Placenta
I	0	0	38	43	0
IIA	30	3	20	31	3
IIB	37	13	35	13	35
IIIA	0	14	0	0	46
IIIB	33	70	7	13	16

WGA. The total activity recovered in each organ was 100%, and the enzyme activity of the five fractions in each ALP preparation is presented as the percentage of the total activity in Table I. A marked difference was found in all fractions among the three isozymes. In fraction II, there was more of fraction IIA, and IIB only in the duodenal ALP. Moreover, there was more of fraction IIIB than IIIA in the liver and the bone ALP, and less in the other isozymes. Fraction IIIA in the term placental ALP accounted for 46% of all the fraction. This high content of fraction IIIA could be a major characteristic of the placental type ALP.

Lectin affinity chromatography of tumour-associated ALPs

We found a unique placental-like ALP in a female patient who died of a malignant disease two months after hospitalization. A high level of the ALP activity was detected in her ascitic fluid and omentum, with a malignant mesothelioma. This ALP was identified enzymologically and immunologically as the tumour-produced placental ALP. These data have been reported elsewhere [25]. The ALP purified partially from her omentum was applied to the lectin column, as were enzyme preparations of normal tissue. As shown in Fig. 2A, the elution profiles differed significantly from that of the term placental ALP in Fig. 1e. There was less of fraction III and more of fraction IIB in the tumour-produced placental ALP than in the term placental ALP. In fraction III, there was less of fraction IIIA and more of fraction IIIB in the tumour-produced placental ALP.

A difference in the lectin-binding affinity between the term placental and the tumour-produced placental ALP might indicate that structural changes in the sugar chains in ALPs are evoked by malignant transformation. To evaluate further the structural changes in tumour-produced ALPs, we studied the high serum ALP activity observed in a male patient who died of a primary hepatoma. The ALP obtained from his liver was applied to the same lectin column as those used for Fig. 2A. The hepatoma ALP was identified enzymologically and immunologically as the tissue-unspecific type of ALP. As shown in Fig. 2B, the Con A elution profiles of the hepatoma ALP also differed from that of the normal liver ALP shown in Fig. 1a; there was more of fraction III in the hepatoma ALP, and this fraction was bound nearly 100% to the WGA column, indicating that only fraction IIIB was increased in the hepatoma ALP.

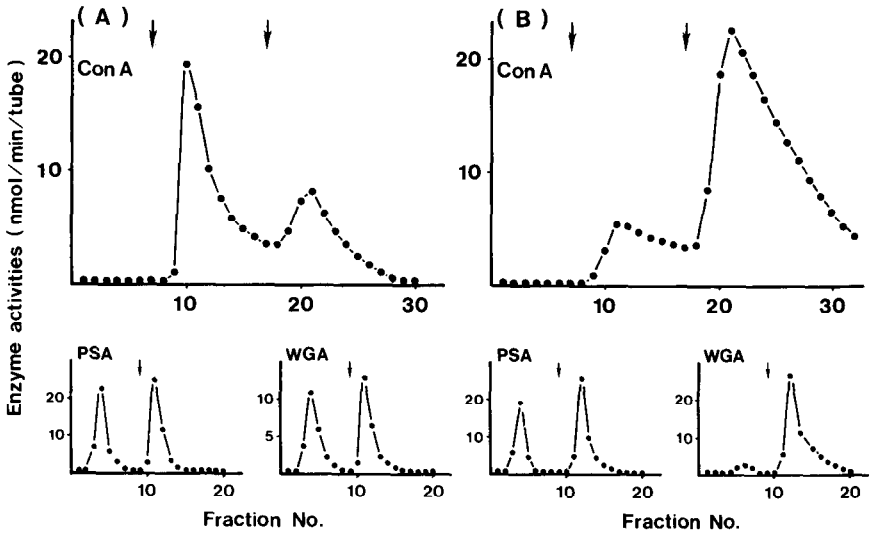

Fig. 2. Lectin affinity chromatography of tumour-associated ALPs. Fractions II and III obtained from the Con A column were applied to a PSA and a WGA column, respectively. (A) Tumour-produced placental ALP; (B) hepatoma ALP. Arrows indicate the starting points of elution with the respective competitive sugars.

The hepatoma ALP seems to share some properties with the tumour-produced placental ALP in lectin-binding affinity: an increase in fraction IIIB and a similar affinity for PSA. Accordingly, the fraction II isozyme forms of the liver, the hepatoma, the term placental and the tumour-produced placental ALP obtained on the Con A column were applied to a PHA-E column. The PHA-E binding affinity of the normal liver ALP was different from that of the term placental ALP, indicating an organ specificity; the fraction bound to the PHA-E column accounted for 35% of the total fraction II in the liver and for 95% in the placental ALP (Fig. 3a and b). On the other hand, affinities of the tumour-produced liver and placental ALPs for PHA-E closely resembled each other (Fig. 3c and d). It is possible that there is a similar differentiation in the sugar-chain structure of the ALP isozyme with malignant transformation.

To investigate further another similarity between the two tumour-associated ALPs, i.e. an increase in fraction IIIB, WGA affinity electrophoresis was performed with the same ALP preparations as those used for lectin affinity chromatography (Fig. 4). On conventional electrophoresis, differences in mobility were observed between the liver and the hepatoma ALPs and between the term placental and the tumour-produced placental ALPs, which would be dependent on the difference in charge in the respective ALP molecules. In the presence of WGA, the mobilities of all preparations tested were slightly or markedly slower than on the conventional electrophoresis, although part of the tumour-associated ALPs remained at the point of origin, indicating that tumour-associated ALPs have a stronger affinity for WGA than do the normal liver or the term placental ALP. Similar results were also obtained with tested all-serum ALPs from certain

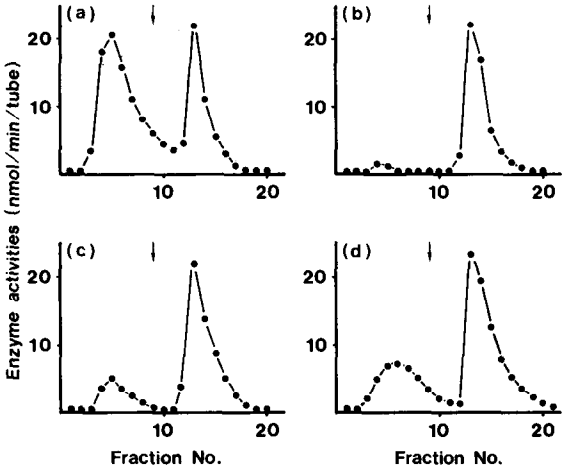

Fig. 3. PHA-E affinity chromatography of fraction II from the Con A column. Fraction II obtained from the Con A column in (a) liver, (b) term placental, (c) hepatoma and (d) tumour-produced placental ALPs was applied to a PHA-E column. The arrows indicate the starting points of elution with GlcNAc.

tumour patients (data not shown). However, the fact of these isozymes remaining at the point of origin may be due to the self-polymerizing character of tumour-associated ALP molecules by differential processing of the enzyme protein precursor, as indicated by others [22,26], or to the disordered processing of sugar chains for the tumour-associated ALP molecules, as reported with the tumour-

Enzyme sources	Treatments	Addition of WGA
Normal liver	non	–
	non	+
Primary hepatoma	non	–
	non	+
	Subtilisin	+
	Endo-F	+
Term placenta	non	–
	non	+
Malignant mesothelioma of omentum	non	–
	non	+
	Subtilisin	+
	Endo-F	+

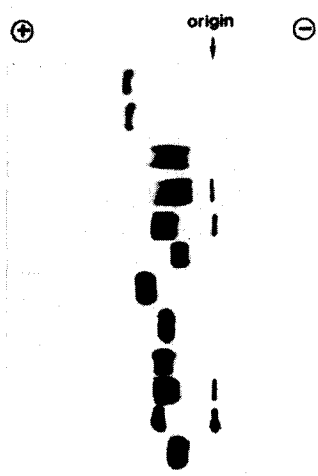

Fig. 4. Zymograms of WGA affinity electrophoresis of ALPs. WGA affinity electrophoresis was carried out using a cellulose acetate plate immersed in a WGA solution as described in the text. Lanes non-added with WGA represent conventional electrophoresis. Tumour-produced ALPs, which remained at a point of origin in the presence of WGA, were treated with subtilisin or endo-N-acetylglucosaminidase F (Endo-F), and run as non-treated ALP.

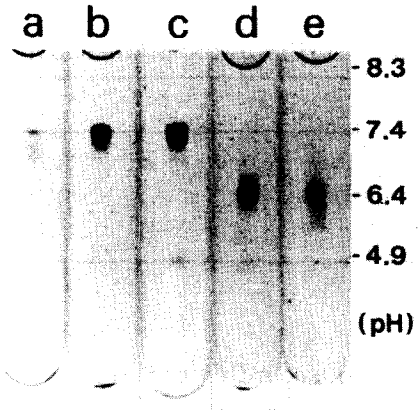

Fig. 5. Isoelectric focusing patterns of the normal and the tumour-associated ALPs. ALPs in (a) serum, (b) liver, (c) hepatoma, (d) term placenta and (e) malignant omentum were focused as described in the text. The isoelectric point was measured with a p*I* marker loaded simultaneously.

produced γGTP [5]. Then, tumour-associated ALP preparation was treated with certain enzymes (Fig. 4). The treatment with subtilisin, which hydrolyses the C-terminal hydrophobic region of protein core [14], led the tumour-associated ALP to remain at the origin, indicating that the above phenomenon cannot be thought of simply in terms of a membrane anchoring moiety on the ALP molecules [19,27]. On the other hand, after treatment with endo-N-acetylglucosaminidase F, which hydrolyses di-N-acetylchitobiose region in biantennary, mannose-rich and hybrid-type sugar chain(s) [15], the enzyme at the origin completely disappeared and was detected in the slower β region of serum protein, suggesting that the enzyme at the origin could be due to the altered sugar structure on the tumour-associated ALPs.

Nature of the protein moiety for normal and tumour-associated ALPs

The results of the lectin-binding affinity shown in Figs. 2–4 indicate structural changes in the sugar chains of ALP molecules with malignant transformation. Since changes in protein moieties may also occur in these cases, isoelectric focusing analysis of ALPs was performed by using preparations treated with PI-specific PLase C and neuraminidase, in order to eliminate the charge modifications by membranous lipids or sialic acids (Fig. 5). Serum ALP (lane a) was simultaneously loaded, as described by other workers; the major isoelectric points of serum ALP were identical with those of the normal liver ALP. These were focused at pH 7.2 and 7.4 for the normal liver and pH 6.4 for the term placental ALP. These data were consistent with those for the tumour-produced liver and placental ALPs, respectively.

We determined the N-terminal amino acid sequence of tumour-associated ALPs using preparations purified further by immunoaffinity chromatography (Table II). From the results, the N-terminal amino acid of the hepatoma ALP was identified as the same as that of normal liver ALP, reported in a previous paper [19]. That of the tumour-produced placental ALP was also the same as the placental

TABLE II

N-TERMINAL AMINO ACID SEQUENCE OF NORMAL AND TUMOUR-ASSOCIATED ALPs

Enzyme source	Sequence
Normal liver★	H-Leu-Val-Pro-Glu-
Hepatoma	H-Leu-Val-Pro-Glu-
Placenta★★	H-Ile-Ile-Pro-Val-
Omentum	H-Ile-Ile-Pro-Val-

★ Data from ref. 19.
★★ Data from ref. 27.

ALP described by Millán [27]. On the other hand, according to the results of one-dimensional peptide mapping for normal liver and hepatoma ALP (Fig. 6), the molecular weights of the respective ALPs were found to be 77 000 for the major and 80 000 for the minor bands for hepatoma, and 80 000 for normal liver. After V8 protease treatment, there were at least five fragments of identical size for two ALPs. Evidence of identical N-terminal amino acid sequence and peptide mapping indicates that there is no difference in primary protein structure between the normal and the tumour-associated ALPs.

DISCUSSION

Studies of altered ALP forms as tumour markers concerned mainly the heat stability of the molecules, the inhibition of the enzyme activity by L-leucine, elec-

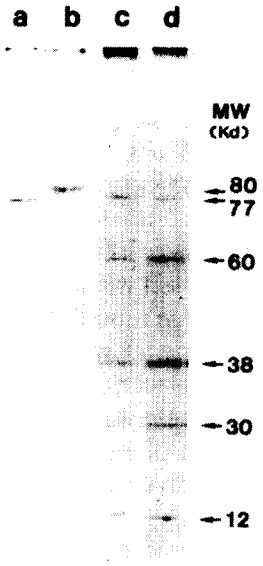

Fig. 6. Peptide map of normal liver and hepatoma ALPs. The hepatoma (a and c) and the normal liver ALP (b and d), ^{32}P-labelled at the active site, were partially digested by V8 protease and electrophoresed in the presence of SDS as described in the text. Lanes a and b and lanes c and d indicate the preparation before and after V8 proteolysis, respectively. Numbers represent molecular weights.

trophoretic mobility or antigenic identification [28]. Many variant forms of oncofoetal and tumour-associated ALPs, e.g. Regan [1], Nagao [2], Kasahara [29] and first-trimester enzyme [30], have been studied. However, whether or not there are structural differences between the normal and the tumour-associated ALPs is still obscure.

We previously established the sugar-chain heterogeneity of rat ALP isozymes by using the serial lectin affinity technique [11]. In summary, differences in lectin-binding affinity were observed not only between the genetically altered ALP isozymes but also among liver, bone and kidney ALPs. Mutual separation among these enzymes seemed to be dependent on structural differences between the sugar chains in the glycoproteins, because in the rat duodenal ALP there was less of the sugar chain that is presumed to be the hybrid type. However, when rat duodenum was treated in vitro with swainsonine, which inhibits α-mannosidase II and leads to the accumulation of hybrid type in the processing pathway of the sugar chain [31], the hybrid-type fraction of duodenal ALP was increased significantly as expected. These findings indicate that intact ALP forms were separated into possibly different types of sugar chain by the difference in their affinity for lectins.

In the present study, we investigated further the sugar-chain heterogeneity in human ALPs from various organs by means of the serial lectin affinity technique. Consequently, elution profiles of human liver, bone, kidney and duodenal ALPs on lectin columns were in good agreement with those of rat ALPs obtained from the corresponding organs, as reported previously [11]. The sugar structures in rat, mouse and bovine γGTP reported by Yamashita and co-workers [5,32] indicate a specificity for the respective organs; the liver γGTP contains mainly the biantennary complex type and the kidney γGTP contains not only the biantennary complex type but also the multiantennary and/or bisecting complex type. In the present study, the sugar-chain heterogeneity in ALP isozymes is probably dependent on the respective organs, as has been reported for γGTP [5,32]. Accordingly, ALPs in the various organs could be separated as differences of the sugar-chain heterogeneity by the serial lectin affinity technique, which will be a useful tool for the identification of the normal ALP forms.

The five fractions isolated by this technique are classified as follows: I, multiantennary or bisected complex-type sugar chains; IIA, biantennary complex type without the internal fucose residue; IIB, biantennary complex type with the internal fucose residue, IIIA, high mannose type or hybrid type with the internal fucose residue; and IIIB, hybrid type without the internal fucose residue [4,33]. Considering structural differences in the sugar chains in human ALPs on the basis of this classification, a significant difference was observed among the three isozymes (Fig. 1, Table I). In particular, there was ca. 50% of fraction IIIA in the term placental ALP, of which there was little, if any, in the other ALP isozymes. Although the bone ALP also possessed this fraction, fraction IIIB was the major component of bone ALP. Placental ALP possessing the high mannose-type or the fucose-bound hybrid-type sugar chain, alongside another property such as specific inhibition of the ALP activity by L-phenylalanine, makes it unique among mammalian ALPs. In addition, the sugar-chain structure in the kidney ALP dif-

fered significantly from those of the liver and bone ALPs. The Con A binding affinity of kidney ALP was similar to that of the intestinal type ALP, and the biosynthetic pathway of the sugar chain(s) may have developed by a similar differentiation in both organs. This would not be a curious phenomenon since the amount of intestinal ALP in the kidney is greater than that in the liver and bone [34].

Furthermore, we observed some important properties of tumour-associated ALPs. The tumour-produced liver and placental ALPs did not have the organ-specific structure in their sugar chain(s), and the structure of both tumour-produced ALPs had two points in common from results of lectin-binding affinities: one is the similar affinity for PSA and PHA-E, and the other is an increase in fraction IIIB type sugar chain, probably the hybrid type without the internal fucose residue type. When fraction II from the Con A column was applied to a PHA-E column, the sugar-chain types of the fraction bound to the PHA-E column were of no known type. However, the similarity in binding affinity for the lectin of the tumour-produced liver and placental ALPs suggests that the two altered ALP forms possess a similar sugar-chain structure, which may depend on the development of golgi complex, such as the ectopic expression and/or deficiency of identical glycosidases or glycosyltransferases in tumour-associated and foetal cells [35,36].

Another similar change in sugar chains due to malignant transformation is an increase in hybrid type without the internal fucose residue, which was also shown in the results of WGA affinity electrophoresis; the mobilities of tumour-associated ALPs in their original organs and sera were retarded, and part of them remained at the point of origin. The tumour-associated ALP at the origin migrated following the endo-N-glucosaminidase F treatment, suggesting that remaining enzyme at the origin would result from the altered sugar structure on the tumour-associated ALPs and that this sugar structure would be more complicated. Rosalki and Ying Foo [37] found a difference between the liver and bone isozymes in human sera by using WGA affinity electrophoresis; the mobility of the bone isozyme was more retarded than that of the liver isozyme, indicating a strong affinity of the bone isozyme for WGA. These results are in good agreement with ours: a large amount of fraction IIIB in the bone ALP in the lectin affinity chromatography. Moreover, the available evidence supports the view that human bone and liver ALPs have identical protein cores [38,39], suggesting that differences between the two types of enzyme, i.e. electrophoretic mobility and heat stability, are due to differences in their sugar chains only. Meanwhile, on polyacrylamide gel electrophoresis with 0.3% Triton X-100, the tumour-associated ALPs migrated a little more slowly towards the anode than the ALPs from normal liver and term placenta. However, they showed an identical mobility after neuraminidase treatment (data not shown). These results suggest that the content of sialic acid in tumour-produced ALPs is less than that in ALPs from normal tissue.

We next investigated the nature of the protein moiety in normal and tumour-associated ALPs (Fig. 5, Table II, Fig. 6). Isoelectric focusing analysis showed that the charge of the ALP molecules was not fundamentally different in normal and tumour-associated enzymes. This was supported by the identical N-terminal

amino acid sequence in both ALPs. Other workers have also reported identical peptide maps and N-terminal amino acid sequence in the placental and tumour-produced placental ALPs [39]. Neuwald and Brooks [22] have reported that the term placental and placental-like ALPs produced by JAR choriocarcinoma cells yielded identical peptide maps after treatment with certain proteases, indicating that the protein core in the placental ALP produced by JAR cells is the same as that in the term placental ALP. The above-mentioned speculation was well supported by the identical peptide mapping also obtained with normal liver and hepatoma ALPs. Although a point mutation by altered splicing of mRNA or a post-translational processing of the enzyme protein [26,40] may take place, the above reports and our present results suggest that the sugar-chain structure in ALPs is modified mainly by malignant transformation. Similar ideas have already been proposed with reference to the slower or faster form of genetically different placental ALPs [41].

Sugar chains in glycoproteins have attracted attention as important regulatory determinants in the secretion of glycoproteins. One report indicated that the accelerated secretion may result from the hybrid-type-rich processing of the sugar chain of the glycoproteins in malignant hepatic cells [42]. The phenomenon that sugar-chain structures of glycoproteins in the hepatoma cells are modified into hybrid type or bypassed from hybrid type to complex type in the sugar processing pathway by inhibition of α-mannosidase II, may be involved in the accelerated secretion of glycoproteins. If so, the secretion might be accelerated in the hybrid-type sugar chains in biosynthetically active cells, not only in the tumour-associated cells.

In the present study, structural changes in sugar chains caused by malignant transformation was demonstrated qualitatively by the serial lectin affinity technique; however, the primary structures of the glycopeptides in ALPs must be known before final identification can be achieved. According to the previous results of sugar compositions, human ALPs had mannose and galactosamine [18]. These data suggest that human ALPs contain both N- and O-glycosidic sugar chains, indicating that structural differences between the N- and O-linked sugar chains of altered ALPs would be an interesting subject for future studies of onco-foetal and tumour-associated glycoproteins.

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