Alkaline phosphatase of mouse teratoma stem cells: Immunochemical and structural evidence for its identity as a somatic gene product

(³²P labeling/immunoprecipitation/two-dimensional electrophoresis/peptide mapping)

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ABSTRACT The immunochemical and structural characteristics of the alkaline phosphatase [orthophosphoric-monoester phosphohydrolase (alkaline optimum), EC 3.1.3.1] from mouse teratoma stem cells derived from the OTT-6050 teratoma (ascitic and solid tumors and the F9 and PCC4 cell lines) have been compared to those of the alkaline phosphatases expressed in normal mouse placenta and several adult organs. Crossreactivity of the stem cell alkaline phosphatase with antisera reacting with placental, kidney, liver, and brain alkaline phosphatases indicated that the stem cell enzyme had common antigenic determinants. Structural studies utilizing two-dimensional electrophoresis of the ³²P-labeled alkaline phosphatase subunits showed that the stem cell, placental, and kidney alkaline phosphatases differed only in their sialic acid content and comigrated after removal of terminal sialic acid by neuraminidase digestion. Furthermore, one-dimensional peptide mapping of partial proteolysis fragments from ³²P-labeled enzymes demonstrated identical fragmentation patterns for the stem cell and somatic enzymes. These immunochemical and structural data indicate that the stem cell alkaline phosphatase is the same core enzyme as that produced in the mouse placenta and kidney, with different amounts of terminal sialic acid. The one mouse alkaline phosphatase examined that differed from the other enzymes was the intestinal alkaline phosphatase. This isoenzyme was not immunochemically crossreactive with the other alkaline phosphatases, did not comigrate in two-dimensional electrophoresis after neuraminidase digestion, and did not give identical peptide maps after partial proteolysis.

Alkaline phosphatase [orthophosphoric-monoester phosphohydrolase (alkaline optimum), EC 3.1.3.1] has been the subject of a number of studies as a biochemical marker for the totipotent stem cells of the mouse teratoma. Damjanov *et al.* (1) first cited the enzyme as a histochemical marker for these cells. Bernstine *et al.* (2) showed that the proportion of stem cells in cell lines derived from the mouse teratoma cell line OTT-6050 could be correlated with alkaline phosphatase specific activity. The use of the stem cell alkaline phosphatase as a specific marker for the totipotent state of the stem cell would be dependent on the uniqueness of this gene product to the stem cell population. Based on the behavior of the enzymes in thermal inactivation and D-phenylalanine inhibition studies by Bernstine *et al.* (2), the stem cell enzyme was found to resemble the isoenzymes of mouse kidney and placenta but differed from the isoenzymes of mouse liver and intestine. Because functional studies on crude cell extracts are not adequate as a sole criterion for determining differences between gene products, Wada *et al.* (3) compared the enzymes of the ascitic and solid tumor forms of OTT-6050 with those of mouse kidney and placenta by using sodium dodecyl sulfate (NaDodSO₄)/polyacrylamide gel electrophoresis and found that solid tumor and ascitic tumor

enzymes had a subunit molecular weight of 73,000 and differed from those obtained for kidney (71,000) and placenta (82,000). Electrophoresis in the absence of NaDodSO₄ also indicated differences between the stem cell and kidney and placental enzymes. Subunit molecular weights and electrophoretic mobilities demonstrated differences in the size and charge properties of the stem cell enzyme and the kidney and placental isoenzymes; however, it was recognized that these differences could be caused by differences in post-translational glycosylation rather than by the expression of different gene products.

To clarify these conflicting sets of experimental data, we have purified mouse placental alkaline phosphatase (PAPase), produced an antisera against it, and studied the immunocross-reactivity of the alkaline phosphatases found in various mouse tissues and solid and ascitic tumors and in the *in vitro* cell lines F9 and PCC4 (4), all derived from the mouse teratoma line, OTT-6050. These studies were expanded by using two-dimensional electrophoresis and one-dimensional peptide mapping of the teratoma stem cell enzyme and the kidney (KAPase), PAPase, and intestinal (IAPase) isoenzymes. Our results indicate that the stem cell alkaline phosphatase is not a gene product unique to stem cells and suggest why there has existed an apparent conflict among previously reported results.

METHODS

Alkaline Phosphatase Preparations. Stem cell alkaline phosphatase was solubilized from microsomal pellets obtained from F9 and PCC4 cells. The cells were grown at 37°C in sealed 490-cm² plastic roller bottles (Corning) by using minimal essential medium supplemented with 10% fetal calf serum and a 1:100 dilution of antibiotic/antimycotic solution (GIBCO). The pH was maintained with 25 mM Hepes (pH 7.2). The cells were washed free of medium with isotonic NaCl, scraped into 0.25 M sucrose/5 mM NaHCO₃, pH 8.1/1 mM phenylmethylsulfonyl fluoride (PMSF), and homogenized in a Potter-Elvehjem homogenizer. Nuclei and mitochondria were pelleted at 1000 × *g* for 10 min and 27,000 × *g* for 7.5 min prior to pelleting of microsomes at 78,000 × *g* for 100 min. The microsomal pellet was extracted with 2% Triton X-100 (scintillation grade, New England Nuclear)/5 mM NaHCO₃, pH 8.1/1 mM PMSF for 5 min at room temperature. The extract was cooled at 4°C and centrifuged at 138,000 × *g* for 60 min. The enzyme preparation was stored at -20°C.

Stem cell alkaline phosphatase was partially purified from serially transplanted solid and ascitic tumors derived from OTT-6050, by using the same tumors and methods described by Wada *et al.* (3).

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Abbreviations: NaDodSO₄, sodium dodecyl sulfate; PAPase, placental alkaline phosphatase; IAPase, intestinal alkaline phosphatase; KAPase, kidney alkaline phosphatase; PMSF, phenylmethylsulfonyl fluoride.

Alkaline phosphatase from liver and IAPase were prepared by Triton X-100 extraction of plasma membrane-enriched fractions from tissues of 129J adult females. Liver plasma membranes were obtained by the method of Aronson and Touster (5). Brush border membranes from the small intestines were prepared by the method of Miller and Crane (6), with an added final step in which the membranes were floated out of 41% sucrose by centrifugation at $112,000 \times g$ for 90 min. These membranes were extracted with Triton X-100 as described for the F9 and PCC4 microsomes. ZnCl_2 (1 mM) was added to the Triton extracts of intestinal membranes because the membrane preparation in EDTA buffer produced an apoenzyme that must be reactivated with Zn^{2+} .

KAPase and PAPase were partially purified by the method of Sussman *et al.* (7). This method used butanol extraction to solubilize the enzyme, DEAE-cellulose anion exchange chromatography, and gel filtration in Sephadex G-200 to purify the enzyme. Kidneys were obtained from 129J adult females; placentas (F_1 [129/J $\times$ C3H/HeJ]) were obtained from late gestational, pregnant 129J females. Alkaline phosphatase was assayed by the method of Bessey *et al.* (8), which follows the cleavage of *p*-nitrophenyl phosphate to *p*-nitrophenol by absorbance at 405 nm. One unit of enzyme activity is defined as 1 μmol of product per min at 37°C .

Preparation of Antiserum to Mouse PAPase. Antiserum was raised against mouse PAPase by injection of partially purified enzyme emulsified with Freund's complete adjuvant (GIBCO) at four sites into both axilla and groin areas of a young female goat. The first injection contained 80 μg of enzyme. The second injection was administered 4 weeks later and contained the same amount of enzyme. Two weeks after the second injection the animal was bled for antiserum.

Immunoprecipitation Assay. The mouse isoenzymes of alkaline phosphatase were tested for crossreactivity with anti-PAPase antiserum by second antibody precipitation of the antigen-antibody complex as described by Sussman *et al.* (7). Unprecipitated enzyme was assayed in the supernatant after the immune precipitate was pelleted.

Two-Dimensional Electrophoresis of ^{32}P -Labeled Alkaline Phosphatases. In order to facilitate detection of the subunits of mouse alkaline phosphatase in two-dimensional electrophoretic maps, the enzymes were active-site-labeled with $^{32}\text{PO}_4$ according to Milstein (9), as described by Wada *et al.* (3). The KAPase, PAPase, and teratoma alkaline phosphatase were immunoprecipitated with anti-PAPase prior to ^{32}P labeling. The IAPase was directly labeled, without immunoprecipitation. The labeled enzyme, which was prepared in the form of an acetone precipitate, was dissolved in 100 μl of 8 M urea/2% Triton X-100/5 mM NaPO_4 , pH 8.1/1 mM PMSF. 2-Mercaptoethanol was added to 5% (vol/vol), and the solution was incubated at room temperature for 10 min prior to analysis by two-dimensional electrophoresis.

Two-dimensional electrophoresis was conducted according to Wada *et al.* (10), which was a modification of the O'Farrell system (11). This system utilizes isoelectric focusing to resolve urea-denatured subunits in the first dimension and NaDodSO₄/polyacrylamide slab gel electrophoresis in the second dimension. Autoradiography of the dried gels as described (10) was used to detect the ^{32}P -labeled enzyme subunits.

Two-dimensional coordinates in the electrophoretic maps were used to estimate the subunit isoelectric points (pI) and molecular weight (M_r) by using standard curves for pI measured in duplicate focusing gels and standard curves for M_r determined with protein standards by the method of Weber and Osborn (12).

Peptide Mapping of ^{32}P -Labeled Alkaline Phosphatase. ^{32}P -Labeled isoenzymes of mouse alkaline phosphatase were subjected to partial proteolysis and NaDodSO₄/polyacrylamide electrophoresis of the resulting peptides according to the method of Cleveland *et al.* (13).

Approximately 0.08 unit of enzyme was ^{32}P labeled as described by Wada *et al.* (3). The ^{32}P -labeled subunit was purified by electrophoresis on an 8% polyacrylamide gel slab containing NaDodSO₄ according to Laemmli (14). The enzyme subunit band was visualized by autoradiography for 1–2 hr on Kodak Royal X-Omat x-ray film and then cut out by using a scalpel. The band, 3–4 mm wide, was cut into four pieces, each containing approximately 0.02 unit of enzyme which represents 80 ng of pure enzyme protein. The enzyme in these gel slices were treated according to the procedure of Cleveland *et al.* (13), which utilized *Staphylococcus aureus* protease (Miles-Yeda Laboratories) to partially digest the protein of interest in the stacking gel of the NaDodSO₄ gel used to analyze the proteolytic peptides. The standard amounts of *S. aureus* protease used on the four gel slices were 0, 20, 200, and 500 ng. Upon completion of the electrophoresis, the gel was fixed in 10% trichloroacetic acid for 1 hr, 7% (vol/vol) acetic acid/3% (vol/vol) glycerol overnight, and fresh acetic acid/glycerol for 1 additional hr. The gel was then dried and autoradiographed for 3–7 days at -76°C with a Dupont Lightning Plus intensifying screen and preflashed film.

Trypsin (Worthington) was also used for partial proteolysis of the ^{32}P -labeled phosphatase subunits. The trypsin was used in the same manner as the *S. aureus* protease and found to be effective at 5.0 μg .

Neuraminidase Digestion of the Alkaline Phosphatase Isoenzymes. Two-dimensional electrophoresis and peptide mapping of neuraminidase-digested alkaline phosphatases were done. Prior to ^{32}P labeling, 0.08 unit of alkaline phosphatase was incubated with 5 μg of *Clostridium perfringens* neuraminidase (Worthington) and 1 mM PMSF to inhibit endogenous proteases. The incubation was conducted in a reaction volume of 80 μl of 0.05 M Na acetate (pH 5.6) at 37°C for 10 min and was stopped by cooling at 0°C on ice. This incubation mixture was directly labeled with $^{32}\text{PO}_4$ as described above and subsequently subjected to electrophoresis and peptide mapping.

RESULTS

Immunoprecipitation of Mouse Alkaline Phosphatases with Anti-PAPase Antiserum. The antiserum to mouse PAPase was found to have a high titer against PAPase. Titration curves indicated that 1.0 μl of antiserum precipitated 0.077 unit of PAPase, approximately 110 ng, at 50% antigen excess. At 3-fold antibody excess, 100% of the PAPase was precipitated. Testing for crossreactivity with human PAPase and human liver alkaline phosphatase showed no precipitation of these enzymes by mouse anti-PAPase at antibody excess.

When the mouse isoenzymes from kidney, liver, brain, and intestine and the stem cell enzymes from F9, PCC4, solid tumors, and ascitic tumors were tested for crossreactivity, all of these phosphatases precipitated with anti-PAPase except for the IAPase (Table 1). The lack of immunoprecipitation of the IAPase could not be attributed to the presence of proteases or other interfering substances in the IAPase preparation because mixing experiments demonstrated that the KAPase could be immunoprecipitated quantitatively from a solution containing both KAPase and IAPase.

Two-Dimensional Electrophoresis of ^{32}P -Labeled Isoenzymes. Two-dimensional electrophoresis of PAPase and KAPase by utilizing isoelectric focusing in polyacrylamide gel for the first dimension and NaDodSO₄/polyacrylamide gel elec-

Table 1. Immunoprecipitation of mouse alkaline phosphatases with anti-PAPase

Isoenzyme	Enzyme remaining in supernatant*		
	Control	Anti-PAPase	% precipitation
Placenta	18.9	0.1	99
Kidney	15	0.4	97
Liver	15.4	1.2	92
Brain	13.7	0.3	98
Intestine	39.4	37.2	6
F9	21.1	0.3	98
PCC4	24.0	0.3	99
Solid tumor	22.7	0.4	98
Ascites	20.2	0.1	100
Mixing experiment: Kidney (61%) + intestine (39%)	22.4	9.8	56

* Enzyme activity is expressed as units (μmol product per min at 37°C) $\times 10^{-4}$.

trophoresis in the second dimension indicated a difference in both M_r (71,000 for KAPase and 82,000 for PAPase) and mean pI (7.10 for KAPase and 6.14 for PAPase). Close examination of the shape and pattern of the spots in the two-dimensional

maps revealed the presence of multiple spots for each of the enzyme subunits; these varied both in charge and in molecular weight (Fig. 1A). This variation in charge and molecular weight was proportional because the spots were seen to lie along a diagonal line connecting the most acidic, high molecular weight component of PAPase to the least acidic, lowest molecular weight component of KAPase.

Two-dimensional electrophoresis of the stem cell alkaline phosphatases from solid tumor, ascitic tumor, F9 cells, and PCC4 cells all gave subunits that migrated on the diagonal line connecting the PAPase and KAPase subunits. These subunits had a M_r of approximately 73,000 and mean pIs ranging from 6.3 to 7.0, which were between the PAPase and KAPase subunit values in two-dimensional maps. In mixing experiments in which PAPase, KAPase, and F9 phosphatase were electrophoresed together, the F9 phosphatase subunit appeared at the acidic shoulder of the KAPase subunit (Fig. 1B). The PCC4 phosphatase appeared at a pI similar to F9 (data not shown). The solid tumor phosphatase appeared at a more acidic pI than the F9 phosphatase (Fig. 1C), and the ascitic tumor phosphatase appeared at a pI similar to that of the solid tumor (data not shown).

The IAPase had a position in the two-dimensional electropherograms that distinguished it from the other isoenzymes. At a M_r of 71,000 and a mean pI of 6.14, this enzyme subunit

FIG. 1. Two-dimensional electrophoresis of ^{32}P -labeled alkaline phosphatase subunits (A) KAPase and PAPase: 0.03 unit of ^{32}P -labeled, partially purified enzymes were run together in a pH 3–10 gradient isoelectric focusing (IEF) gel rod for the first dimension and electrophoresed in an 8% acrylamide/0.1% NaDodSO₄ gel slab in the second dimension. The dried gel was autoradiographed with a fluorescent screen for 1 day. The mean M_r of KAPase and PAPase are 71,000 and 82,000, respectively (3). (B) F9 alkaline phosphatase: 0.03 unit of ^{32}P -labeled F9 enzyme from a microsomal extract was run together with 0.03 unit of PAPase and KAPase. The dried gel was autoradiographed with a fluorescent screen for 4 days. (C) Intestinal and solid tumor (ST) alkaline phosphatases: 0.008 unit of intestinal enzyme from a brush border membrane extract was ^{32}P labeled; 0.008 unit of partially purified solid tumor enzyme, KAPase, and PAPase were immunoprecipitated and ^{32}P labeled. All four of these enzymes were run together. The dried gel was autoradiographed with a fluorescent screen for 16 hr. The positions of the intestinal and solid tumor enzyme subunits were determined by running individual gels for these enzymes. (D) Neuraminidase-digested intestinal, solid tumor, kidney, and placental alkaline phosphatases: 0.008 unit of intestinal enzyme was incubated with $10\ \mu\text{g}$ of *C. perfringens* neuraminidase at 37°C for 10 min prior to ^{32}P labeling; 0.008 unit of solid tumor, kidney, and placental enzyme were also incubated with neuraminidase under the same conditions prior to immunoprecipitation and ^{32}P labeling. All four of the pretreated enzymes were run together. The dried gel was autoradiographed for 8 hr. The positions of the enzyme subunits were also determined in gels run on individual enzymes.

FIG. 2. *S. aureus* protease peptide maps of mouse alkaline phosphatases: 0.06 unit of KAPase (A), PAPase (B), F9 enzyme (C), and IAPase (D) was incubated with 5 μ g of neuraminidase for 10 min at 37°C prior to 32 P labeling and mapping of the *S. aureus* protease-generated active site-labeled peptides. The enzymes were digested with 0, 20, 200, or 500 μ g of protease (lanes from left to right for each enzyme). The dried gel was autoradiographed with a fluorescent screen for 3 days. Protein standards were used to assign M_r coordinates (human transferrin, 80,000; bovine catalase, 60,000; ovalbumin, 45,000; chymotrypsinogen A, 25,000; avidin, 17,000; and horse heart cytochrome c, 12,400).

was clearly off the diagonal formed by PAPase and KAPase (Fig. 1C). IAPase had the same mean pI as PAPase but had a lower M_r . A characteristic further distinguishing IAPase from PAPase was the fact that IAPase was resistant to neuraminidase digestion whereas PAPase was labile to neuraminidase.

Because glycoproteins can exhibit peripheral heterogeneity due to varying degrees of terminal sialic acid content (15), the effect of neuraminidase on the charge and size properties of the phosphatases was tested. Digestion of these isoenzymes with neuraminidase prior to 32 P labeling and two-dimensional electrophoresis resulted in a marked change in the pI and M_r of the PAPase and stem cell enzyme subunits. The coordinates of neuraminidase-digested PAPase and solid tumor alkaline phosphatase subunits coincided precisely with those of digested KAPase (Fig. 1D). Neuraminidase digestion of the KAPase subunit caused no observable change in migration in two-

dimensional gels, indicating low levels of terminal sialic residues in this isoenzyme. The KAPase subunit did not migrate as a single spot, even after neuraminidase digestion, which suggests some charge microheterogeneity not due to terminal sialic acid. The IAPase subunit was unaffected by neuraminidase (Fig. 1D), which is consistent with its reported lack of sialic acid residues (13).

Peptide Mapping of 32 P-Labeled Alkaline Phosphatases. Peptide mapping of the crude and partially purified enzyme preparations was accomplished by a combination of specific 32 PO₄ labeling of the alkaline phosphatase active site, a purification step using NaDodSO₄/polyacrylamide gel electrophoresis, and partial proteolysis of the labeled enzyme subunits by either *S. aureus* protease or trypsin. The proteolytic fragments were separated by electrophoresis in 14% polyacrylamide gel slabs containing NaDodSO₄ and detected by autoradiography. Only the peptides containing active site are detected in this system, enabling peptide mapping of the impure enzymes.

The *S. aureus* protease peptide maps of PAPase and KAPase were similar in pattern, although the first six peptides of PAPase were slightly larger in M_r . Neuraminidase digestion prior to peptide mapping eliminated these differences, resulting in identical maps for PAPase and KAPase (Fig. 2). *S. aureus* protease peptide maps of neuraminidase-digested PCC4 and F9 phosphatases were identical to those of PAPase and KAPase (data for PCC4 not shown). IAPase was the only isoenzyme examined that gave a different map. This isoenzyme gave only four fragments, whereas the other isoenzymes gave nine major fragments. These four IAPase fragments did not directly correspond to any of those of the other isoenzymes.

Because of the resistance of IAPase to *S. aureus* protease, another proteolytic enzyme, trypsin, was used. This enzyme also produced corresponding peptide fragments of KAPase, PAPase, and F9 enzyme after neuraminidase digestion (data not shown). Trypsin was able to digest IAPase; however, there was no direct correspondence between fragments from IAPase and the F9 enzyme (Fig. 3).

DISCUSSION

Studies with human liver alkaline phosphatase and PAPase have demonstrated that the antigenic determinants on these isoenzymes are different and not dependent on the presence of peripheral sialic acid residues (16). These two isoenzymes were also shown to have different primary structures by tryptic peptide mapping, which was consistent with their lack of antigenic crossreactivity. Immunoprecipitation of the mouse kidney, placental, liver, and brain alkaline phosphatases by anti-PAPase antiserum indicated that many of the somatic mouse alkaline phosphatases share antigenic determinants and may be closely related in their primary structure. The immunoprecipitation of the stem cell alkaline phosphatase with anti-PAPase suggested that this alkaline phosphatase also may be structurally related to the other somatic phosphatases. The only mouse phosphatase that did not crossreact with mouse anti-PAPase was IAPase. The complete lack of immunoprecipitation indicated no shared antigenic determinants, which is good evidence that IAPase represents a structurally different enzyme.

Because immunochemical crossreactivity does not imply identical gene products, further structural data were sought to establish the relationship between the somatic enzymes and the stem cell enzyme. Two-dimensional electrophoretic characterization of PAPase, KAPase, and the stem cell enzyme subunits indicated differences in charge and subunit molecular weight. These differences had been previously detected by

FIG. 3. Tryptic peptide maps of IAPase and F9 alkaline phosphatase: 0.08 unit of IAPase (lane a) and F9 alkaline phosphatase (lane b) were incubated with 5 μ g of neuraminidase for 10 min at 37°C prior to 32 P labeling. The 32 P-labeled subunits were purified in an 8% polyacrylamide gel containing NaDodSO₄ and then trypsin digested in the stacking gel of a 14% polyacrylamide gel. The dried 14% gel was autoradiographed with a fluorescent screen for 18 hr (Left) or 4.5 hr (Right).

one-dimensional polyacrylamide gel electrophoresis in the presence and absence of NaDodSO₄ (3). In the present study, these differences in charge and size were entirely eliminated by digestion with neuraminidase, indicating that peripheral sialic acid content was the cause of these differences. The distribution of all mouse phosphatases except IAPase along the diagonal defined by KAPase and PAPase in two-dimensional gels was consistent with the following explanation for the different forms of alkaline phosphatase. Increased sialylation of the subunits was associated with larger molecular weight and more negatively charged forms in two-dimensional electropherograms from the smallest, most basic KAPase subunit up to the largest, most acidic PAPase subunit. The core desialylated enzyme subunit appears to be identical for KAPase, PAPase, and the stem cell enzyme by two-dimensional electrophoresis. Hiwada and Wachsmuth (17) have found that the microheterogeneity of pig KAPase was due to varying degrees of sialylation, and neuraminidase reduced the different forms of the enzyme to a single, desialylated enzyme. In the present study, the desialylated mouse KAPase subunit gave more than one spot in two-dimensional electrophoresis. This may indicate that terminal sialic acid residues are not the sole determinants of the microheterogeneity of mouse KAPase.

Peptide mapping studies were conducted with alkaline phosphatases that were labeled at the active site with ³²P₄. This allowed proteolytic peptide mapping of enzymes that were not purified to homogeneity, because only peptide fragments containing the enzyme active site would be detected by autoradiography. Partial hydrolysis by *S. aureus* protease or trypsin generated a series of active site peptides with different molecular weights, giving characteristic patterns when electrophoresed in NaDodSO₄/polyacrylamide gels. These one-dimensional peptide mapping experiments gave identical patterns for desialylated KAPase, PAPase, and stem cell alkaline phosphatase. This result indicates that the sequences of aspartate, glutamate, lysine, and arginine residues are identical in the polypeptides of these enzymes and that the carbohydrate moieties are of the same size and linked to the same residues of the polypeptide. Thus, the core desialylated enzymes appear to be structurally identical.

IAPase was the only mouse alkaline phosphatase that gave nonidentical one-dimensional peptide maps. With *S. aureus* protease, which hydrolyzes peptide bonds at aspartate and glutamate residues, proteolysis of IAPase was blocked after only three cleavages of the polypeptide, whereas the other isoenzymes were cleaved at a minimum of eight locations. Even higher levels of protease (1 μg) could not force the digestion. Trypsin, which cleaves at lysine and arginine residues, was able to digest the IAPase subunit completely and produced a map that was not identical to the other enzymes. These results indicate that the IAPase core subunit was not identical to that of the other mouse alkaline phosphatases. This difference in the core subunit was also indicated by the two-dimensional electropherograms, which showed the IAPase subunit to be off the diagonal defined by PAPase and KAPase. Furthermore, the acidic pI of IAPase could not be explained by high sialic acid content because this mouse isoenzyme does not contain sialic acid (18).

The difference in the core IAPase subunit could be due to differences in glycosylation, other than terminal sialylation as well as to differences in amino acid sequence. However, post-translational glycosylation alone cannot account for all of the

different properties of the IAPase subunit. This subunit does not share antigenic determinants with the other mouse alkaline phosphatases, and the lack of any immunochemical cross-reactivity suggests differences in polypeptide structure.

The structural data together with the immunochemical data now provide good evidence that the stem cell alkaline phosphatase of the mouse OTT-6050 teratoma is coded for by the same gene that codes for PAPase and KAPase and perhaps for the liver and brain alkaline phosphatases which were only examined immunochemically. This same line of structural and immunochemical evidence suggests that IAPase is coded for by a different gene. These conclusions are partially in accordance with the results of Bernstine *et al.* (2) who found that, by the criteria of heat inactivation and phenylalanine inhibition, the stem cell enzyme resembled PAPase and KAPase but differed from IAPase and the liver phosphatase. In the light of the present study, it can be concluded that the stem cell alkaline phosphatase does not represent a gene product unique to the stem cell population, as appears to be the case for the F9 antigen described by Artzt *et al.* (19). Consequently, this alkaline phosphatase cannot be considered to be a specific marker for the teratoma stem cells. IAPase, on the other hand, appears to be a distinctly tissue-specific gene product and may prove to be of value in studies of intestinal differentiation in the mouse teratoma system.

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