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DIMERIC NATURE AND AMINO ACID COMPOSITIONS OF HOMOGENEOUS CANINE PROSTATIC, HUMAN LIVER AND RAT LIVER ACID PHOSPHATASE ISOENZYMES

SPECIFICITY AND pH-DEPENDENCE OF THE CANINE ENZYME

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Summary

Two isoenzymes of rat liver acid phosphatase (orthophosphoric-monoester phosphohydrolase (acid optimum) EC 3.1.3.2) have been purified to homogeneity, at least one of these for the first time. Both of the rat liver isoenzymes have identical specific activities towards *p*-nitrophenyl phosphate. Molecular weights of the native enzymes are 92 000 for rat liver isoenzyme I and 93 000 for isoenzyme II, while the subunit molecular weights are 51 000 and 52 000, respectively. Data on substrate specificity and pH dependence are presented for the homogeneous canine prostatic enzyme, which is also isolated as a dimeric enzyme of (native) molecular weight 89 000. Carbohydrate analysis data are presented for canine prostatic acid phosphatase and it is further noted that both isoenzymes of rat liver acid phosphatase are also glycoproteins. The amino acid compositions of the two rat liver isoenzymes are presented together with those of the similar dimeric acid phosphatase of human liver and of canine prostate. Comparison of these results with published data for the amino acid composition of human prostatic acid phosphatase shows substantial similarities. However, significant differences are seen in the amino acid composition of rat liver acid phosphatase isoenzyme I as compared to a previous literature report. Most notably, 17 histidine residues are found per mol of isoenzyme I and 18 for isoenzyme II.

Studies of acid phosphatase isoenzymes are of increasing interest because of their involvement in physiological and pathological catabolic processes. The purification of rat liver acid phosphatase (orthophosphoric-monoester phospho-

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hydrolase (acid optimum), EC 3.1.3.2) has been carried out by a number of workers [1—3]. Chromatographic separations of an aqueous homogenate of rat liver obtained following high speed centrifugation showed three separate bands of enzyme activity [4]. Igarashi et al. [1] observed two isoenzymes of acid phosphatase from rat liver and reportedly purified isoenzyme I to homogeneity. They reported the presence of one active site and a single histidine residue per molecule of rat liver acid phosphatase, and a molecular weight of 100 000 [5]. It seemed to us unlikely that this molecule contained a single residue of histidine. First, collected data [6] for the average composition of proteins would suggest that a protein of this size should contain, on the average, about 16 histidine residues. Secondly, structural and mechanistic data obtained by us and by others in studies on related acid phosphatases show that the rat liver enzyme merited reinvestigation: a molecular weight of 102 000 has been reported for human prostatic acid phosphatase [7], and it is known to be a dimer [8] which may be dissociated into two equivalent subunits [9] of mol. wt. 49 000—55 000. We have recently isolated and purified to homogeneity an acid phosphatase isoenzyme from human liver. It has a molecular weight of 92 000 and is also a dimer as evidenced by SDS polyacrylamide gel electrophoresis (unpublished data). (A molecular weight of 107 000 had previously been reported for the partially purified enzyme [10]). Therefore, in view of the molecular properties of acid phosphatases from human prostate and human liver, we reinvestigated the rat liver enzyme. In the course of this study we purified the second isoenzyme to homogeneity for the first time. The subunit molecular weights and amino acid compositions of both rat liver phosphatase isoenzymes have been determined. We also describe kinetic and structural properties of homogeneous canine prostatic acid phosphatase, an enzyme which was studied only with partially purified preparations [11,12]. A preliminary communication of part of our results has been presented [13].

Materials and Methods

Enzyme assay and kinetic studies. The activity of both isoenzymes of rat liver acid phosphatase was measured by incubating 10—50 µl diluted enzyme for 5—10 min at 37°C with 2.54 mM *p*-nitrophenyl phosphate (Sigma Chemical Co.) in a total volume of 0.5 ml 100 mM sodium acetate (pH 5.0). The reaction was stopped by addition of 1.5 ml 0.25 M NaOH. The absorbance of the liberated *p*-nitrophenolate ion was read at 410 nm. One unit of enzyme catalyses the hydrolysis of 1 µmol *p*-nitrophenyl phosphate per min and the specific activity is the number of enzyme units per mg enzyme protein. Protein was determined [14,15] using crystalline bovine serum albumin as standard.

Homogeneous canine prostatic acid phosphatase was isolated by affinity chromatography using Sepharose-bound *N*-(6-aminohexyl)tartramic acid as an affinity absorbant [16]. In studies of the pH dependence, assays were conducted at 25°C using either 25 mM 3,3-dimethyl glutarate/NaOH (pH 3.6—7.3) or 25 mM barbital/HCl (pH 7.3—9.0) buffers containing 0.01% Triton X-100, the total ionic strength in each case being adjusted to 150 mM with NaCl. 8—10 substrate concentrations were employed and these ranged from approx. 1/5 to 5 · Km (for pH 3.6 to 7.3), 1/5 to 2 · Km (for pH 8.2) and 1/12 to 1.0 · Km

(for pH 9.0). Triplicate determinations were made at each substrate concentration at a given pH. Enzyme was incubated for 10 min in 2.0 ml buffer containing substrate and the reaction was stopped by adding 0.4 ml 1.25 M NaOH and absorbance was read at 400 nm using a Gilford 2000 spectrophotometer. Values of K_m and V were calculated using Cleland's HYPER computer program [17]. Homogeneous enzyme was used in studies of the pH-dependence and of the substrate specificity. The Michaelis constants for the hydrolysis of *p*-nitrophenyl phosphate by the canine enzyme were determined at 25°C using 50 mM sodium acetate (pH 4.8)/100 mM NaCl. Following incubation for 10 min the reaction was stopped by addition of 1.25 M NaOH and the absorbance at 400 nm was measured. For studies with choline phosphate and β-glyceryl phosphate, the extent of enzymic reaction was determined by measuring the amount of phosphate liberated [18]. Implicit in the present use of this assay system is the fact that molybdate is a very strong competitive inhibitor [19] and indeed a transition state analog [20] for these acid phosphatases. Quantitative determination of liberated P_i was made by measuring phosphomolybdic acid spectrophotometrically at 700 nm ($\epsilon = 4002\ M^{-1} \cdot cm^{-1}$). 25 μl diluted enzyme solution was added to 1 ml substrate in 50 mM sodium acetate (pH 4.8)/100 mM NaCl. Following incubation at 25°C for 10 min the reaction was stopped by adding 2 ml 1% ammonium molybdate in 200 mM sodium acetate (pH 4.0) and 0.2 ml 0.5% ascorbic acid in 200 mM sodium acetate (pH 4.0). After 30 min, the absorbance was read. The enzyme activity with thymophthalein monophosphate as a substrate was assayed using slight modifications of literature procedures [21]. The enzyme was incubated with substrate at 37°C for 10 min in 0.75 ml 200 mM sodium acetate/0.005% Triton X-100 (pH 5.4). The reaction was stopped and the color developed by adding 1 ml freshly prepared 0.1 M Na_2CO_3/0.1 M NaOH. The absorbance was read at 540 nm ($\epsilon = 3.8 \cdot 10^4\ M^{-1} \cdot cm^{-1}$).

Polyacrylamide gel electrophoresis. 9% acrylamide gels at pH 8.9 in 0.37 M Tris/HCl [22.23] were run using a running buffer diluted 1 : 10. The acid phosphatase samples containing 20% sucrose, 6—18 μg protein and 5 μl Bromophenol Blue dye were applied to the gels (8 × 0.5 cm) and electrophoresis was carried out at 3.0 mA/gel for 5 h. The gels were stained for enzyme activity with a solution containing Fast Garnet GBC salt and α-naphthyl acid phosphatase (1 mg of each per ml in 50 mM sodium acetate, pH 5.0). This mixture stains simultaneously with substrate hydrolysis [4]. The protein was stained with Coomassie Brilliant Blue.

Sodium dodecyl sulfate polyacrylamide-gel electrophoresis. The proteins (15—20 μg) were prepared for SDS gel electrophoresis by dissolving them in a solution of 100 μl 1% SDS/2% mercaptoethanol in 0.01 M H_3PO_4; the pH was adjusted to 6.8 with solid Tris, the samples heated at 100°C for 5 min and stored at room temperature overnight prior to electrophoresis [24]. 7.5% acrylamide gels were stacked and run according to the method of Weber and Osborn [25]. The standards used were bovine serum albumin, ovalbumin, α-chymotrypsin, trypsin and cytochrome *c*. In duplicate experiments, correlation coefficients of 0.998 and 0.985 were found for the lines relating the logarithm of the molecular weight with mobility in the standard curves. The molecular weight of isoenzyme I was determined by Sephacryl S-200 chromatography

using ribonuclease, α-chymotrypsin, ovalbumin and bovine serum albumin as standards. Correlation coefficients of 0.999 and 0.998 were obtained for the standard curves in duplicate determinations.

Amino acid analyses. 3 50-μg samples of each isoenzyme were hydrolysed under vacuum in 6 M HCl at 110 ± 2°C for 24, 48 and 72 h. HCl was removed by evaporating to dryness under vacuum, the hydrolysates were dissolved in H_2O and analysed on a Durrum amino acid analyzer. The values for serine, threonine and tyrosine were corrected for decomposition by extrapolation to zero time of the values obtained at 24, 48 and 72 h. The isoleucine value was obtained from the 72-h hydrolysis. Methionine values are the sums of methionine and methionine sulfoxide. Values of other amino acids are the average of the values obtained from three different times of hydrolysis and each value is rounded to the nearest integer. A small peak corresponding to cysteine was observed in the amino acid analysis chromatograms in the case of rat liver isoenzyme I and this may correspond to the value of 2 cysteine residues reported by Igarashi et al. [5].

Purification of rat liver acid phosphatase isoenzymes I and II. Both isoenzyme I and isoenzyme II of rat liver acid phosphatase were isolated by modifications of the method of Igarashi and Hollander [1]. Barely thawed frozen rat livers were homogenised in 50% glycerol. The pH of the supernatant obtained after centrifugation of the homogenate was adjusted to 5.0 with 1 M acetic acid and a resulting precipitate was removed by centrifugation. The supernatant was dialysed against 5 mM sodium acetate (pH 5.0) and a subsequent 40—75% $(NH_4)_2SO_4$ fraction was dialysed against 5 mM imidazole acetate (pH 7.3) for 24 h. The enzyme preparation was applied onto a 4 × 75 cm DEAE cellulose column preequilibrated with 10 mM imidazole acetate (pH 7.3). Two enzyme activity peaks corresponding to isoenzymes I and II were eluted with a linear 0—0.5 M NaCl gradient made up using 1 l buffer and 2 l buffer plus 0.5 M NaCl. $(NH_4)_2SO_4$ precipitates (0—75%) of both pooled activity peaks corresponding to isoenzyme I and isoenzyme II were dissolved in 10 mM sodium acetate (pH 4.8) and dialysed against the same buffer. Preparations of isoenzyme I and isoenzyme II were passed through 3.5 × 36 cm and 2.4 × 45 cm columns of CM-cellulose equilibrated with 50 mM sodium acetate (pH 4.8), respectively. Isoenzyme II was eluted at 252 ml from the CM-cellulose column with 30 mM sodium acetate/2 mM sodium pyrophosphate (pH 5.2). The specific activity was 17—18 units/mg. Chromatography on a 1 × 120 cm Sephacryl S-200 column equilibrated with 20 mM NaCl/50 mM Tris/HCl (pH 7.0) resulted in the elution of a single symmetrical activity band coincident with a protein band. The specific activity was 18—19 units/mg. The enzyme was concentrated and stored in 50 mM sodium acetate (pH 5.0).

The complete purification of isoenzyme I required more steps. Isoenzyme I was eluted from the CM-cellulose column with an elution volume of 450 ml by 50 mM sodium acetate/2 mM pyrophosphate (pH 5.6) after the column had been washed with 30 mM sodium acetate/2 mM pyrophosphate (pH 5.2). The active fractions of isoenzyme I from the CM-cellulose column were concentrated using an Amicon Diaflow PM-30 membrane, dialysed against 5 mM imidazole acetate (pH 6.8) and applied onto a 4 × 34 cm DEAE-cellulose column equilibrated with the same buffer. The column was eluted with a linear 0—0.5

M NaCl concentration gradient in a total volume of 800 ml. The vessel containing the higher salt concentration also had a pH of 4.5, thus constituting a pH gradient from 7.0 to 4.5 superimposed on the salt concentration gradient. The activity peak corresponding to isoenzyme I eluted at 360 ml. It was concentrated, equilibrated against 25 mM sodium acetate/50 mM NaCl (pH 5.5), and then passed through a 2 × 24 cm column of Sepharose 4B-concanavalin A [26] equilibrated with 25 mM sodium acetate/50 mM NaCl (pH 5.5). The column was washed with the starting buffer until the 280-nm absorbance of the eluate was zero. The enzyme was eluted with a linear 0—0.4 M concentration gradient of D-(+)-mannose in total volume of 700 ml. Active fractions were pooled, concentrated to 1 ml and equilibrated with 50 mM Tris/HCl/50 mM NaCl (pH 7.0). The pooled enzyme preparation was further purified by chromatography through a 1 × 101 cm column of Sephacryl S-200 equilibrated with the same buffer. The activity band had an elution volume of 65 ml. The first half of the active fractions were pooled, concentrated to a small volume, dialysed against 10 mM sodium acetate (pH 5.0) and applied onto a 2.5 × 39 column of Sepharose-4B-*N*-hexyltartramic acid [16] equilibrated with 10 mM sodium acetate (pH 5.0). The enzyme came off as a sharp symmetrical activity peak fully coincident with a 280 nm absorbance band.

The rationale of separating rat liver acid phosphatase isoenzyme I from isoenzyme II using DEAE-cellulose is that isoenzyme I comes off early in the chromatography [1]. Similarly, in the case of human liver acid phosphatase, an isoenzyme of high specific activity which seems to be analogous to isoenzyme I of rat liver acid phosphatase is eluted early from DEAE-cellulose columns [10], the conditions for elution from DEAE-cellulose columns being almost identical in the two cases. Conversely, isoenzyme II can be eluted first from a CM-cellulose column if the proper conditions are chosen. Brightwell and Tappel [2] had observed similar profiles for two isoenzymes of rat liver acid phosphatase on DEAE- and CM-cellulose columns.

Glycoprotein carbohydrate analyses. The homogeneous acid phosphatase was dialyzed extensively against 10 mM sodium acetate (pH 5.0) and then against water. The protein was lyophilized and a 1-mg sample employed for analysis. Following methanolysis, re-*N*-acetylation and conversion to the trimethylsilyl derivatives [27] the sample was analyzed by gas chromatography on a 6-ft OV-17 column using a temperature program as follows: start, 120°C; raised 4°C/min for 15 min; raised 2°C/min for 15 min and finally raised 5°C/min for 6 min.

Results and Discussion

Homogeneous canine prostatic acid phosphatase exhibits many similarities to the corresponding human enzyme, and this resemblance is reflected in the substrate specificity. Table I gives Michaelis constants for the reaction with several substrates commonly used to assay acid phosphatases. The specific activity measured with *p*-nitrophenyl phosphate (Table I) is approx. 1/7 of that observed with human prostatic acid phosphatase [16], and the activity towards β-glyceryl phosphate is similarly smaller [28]. The K_m values are similar in magnitude to those observed with the human enzyme. As is observed with the

TABLE I

SPECIFICITY OF CANINE PROSTATIC ACID PHOSPHATASE TOWARDS SOME TYPICAL SUBSTRATES

The extent of hydrolysis was measured in acetate buffers at 25°C. Michaelis constants were calculated using the program HYPER [17]. V is expressed as μmol product released per min per mg protein.

Substrate	pH	V	K_m (mM)
p-Nitrophenyl phosphate	5.0	39	0.096
Choline phosphate	4.8	18	8.6
β-Glyceryl phosphate	4.8	42	3.5
Thymolphthalein phosphate *	5.4	20	0.13

* At substrate concentrations higher than 0.25 mM substantial nonlinearity is observed in reciprocal plots or Eadie-Hofstee plots. This behavior is similar to that seen with human liver acid phosphatase [10]. The Michaelis constant given here is the value obtained in the substrate range 0.025—0.25 mM.

human prostatic enzyme [29], but in distinct contrast to the human liver enzyme [10], choline *O*-phosphate is a good substrate for canine prostatic acid phosphatase in that V is high, although K_m is somewhat high (Table I). The hydrolysis of choline phosphate may be related to the natural physiological function of prostatic enzyme since there is a high concentration of choline in the seminal fluid [29].

The pH-dependence of the canine enzyme (Table II) is also generally similar to the human prostatic enzyme. As recently described in detail [28], V is characterized by a broad plateau between pH 3 and 7. The sharp fall-off in enzymatic activity which is typically observed with acid phosphatases assayed at fixed substrate concentrations (particularly when *p*-nitrophenyl phosphate is used as a substrate) is due to an unfavourable substrate ionization: K_m^{app} sharply increases on passing through the second ionization constant of the substrate [28]. This is also seen with the canine enzyme (Table II). Only at high pH (>7) does V decrease, due to the ionization (and consequent decrease in reactivity) of a covalent phosphoenzyme intermediate [30,31].

Two isoenzymes of rat liver acid phosphatase were purified to homogeneity. Both isoenzymes show only one protein band and a corresponding activity band on polyacrylamide gel electrophoresis (Fig. 1). Isoenzymes I and II of rat

TABLE II

pH DEPENDENCE OF THE MICHAELIS CONSTANTS FOR THE HYDROLYSIS OF *p*-NITROPHENYL PHOSPHATE CATALYZED BY HOMOGENEOUS CANINE PROSTATIC ACID PHOSPHATASE

V is expressed as μmol product released per mg protein per min.

Buffer	pH	V	K_m (mM)
3,3-Dimethylglutarate	3.6	39.6	0.052
	4.6	35.1	0.046
	5.4	32.1	0.052
	6.3	45.3	0.38
Barbital	7.3	31.5	2.0
	8.2	14.3	5.9
	9.0	8.96	29.

liver acid phosphatase each have specific activities of 18—19 (at 37° C). A similar specific activity for isoenzyme I had been reported [1]. Replicate determinations of the molecular weight of isoenzyme I using Sephacryl gel chromatography gave values of 91 000 and 93 000; a molecular weight of 93 000 was obtained for isoenzyme II. Thus, the molecular weights of the two isoenzymes are identical. SDS gel electrophoresis also showed only one protein band (Fig. 1), but the molecular weight of isoenzyme I by SDS gel electrophoresis was 51 000 and that of isoenzyme II was 52 000. Similarly a (native) molecular weight of 89 000 and subunit molecular weight of 52 000 was observed with canine prostatic acid phosphatase (Fig. 1). Sucrose gradient density centrifuga-

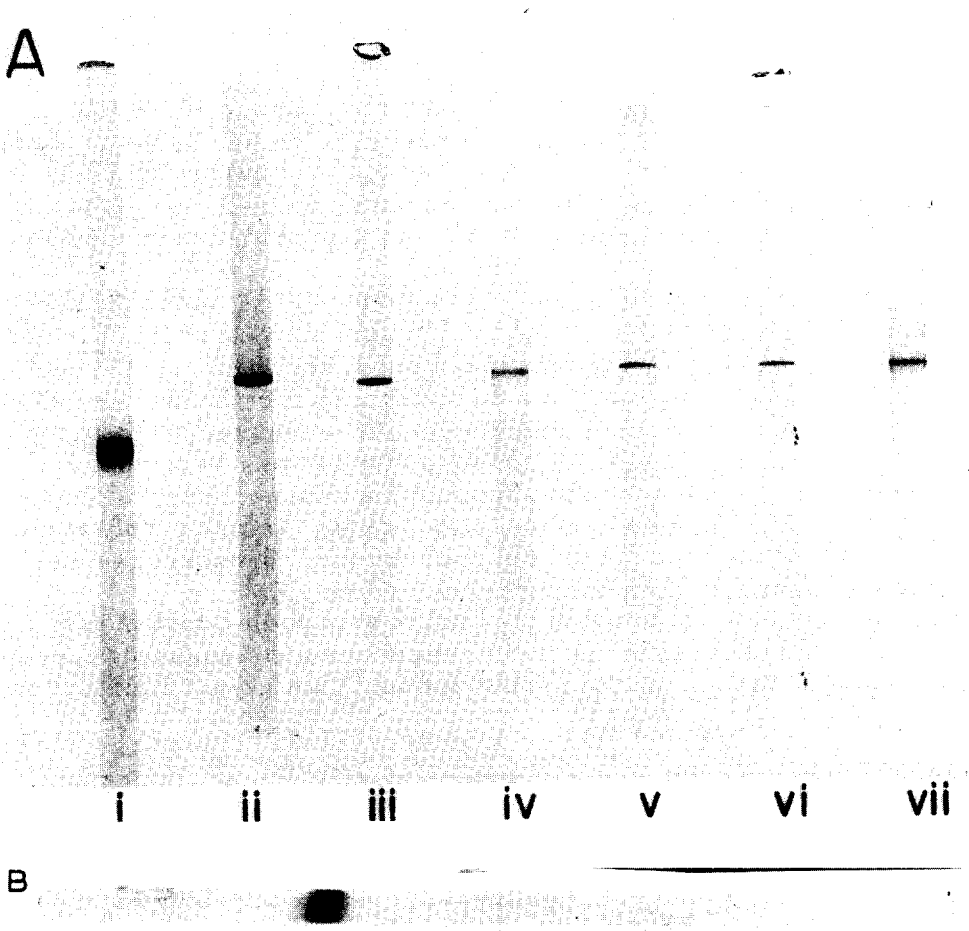

Fig. 1. A. Polyacrylamide gel electrophoresis of purified isoenzyme I and isoenzyme II of rat liver acid phosphatase. i, SDS polyacrylamide gel electrophoresis of isoenzyme I: 15 μg of the denatured protein was placed on the gel and electrophoresis carried out, ii—iv are polyacrylamide gel electrophoresis profiles for isoenzyme I and v—vii for isoenzyme II. ii and iii (14 and 7 μg protein) and v and vi (10 and 5 μg protein) are stained for protein with Coomassie Brilliant Blue. iv and vii are stained for activity with α-naphthyl phosphate-Fast Garnet GBC salt [4]; 7 and 5 μg of protein were used for isoenzyme I and II respectively. The anode is at the top. B. SDS polyacrylamide gel electrophoresis of homogeneous canine prostatic acid phosphatase. 10 μg of protein were employed.

tion afforded a molecular weight of 89—102 · 10^3 for human prostatic acid phosphatase [32]. This enzyme is also known to have two identical subunits as determined by SDS gel electrophoresis [8] and tryptic peptide mapping [9]. Similar results for the molecular weight and subunit composition have been obtained by us in the case of human liver acid phosphatase (unpublished data). Thus, all of the isoenzymes discussed here are dimeric enzymes made up of equivalent subunits.

Table III shows the amino acid composition of isoenzyme I (together with previously reported data [5]), isoenzyme II, human [7] and canine prostatic acid phosphatases, and human liver acid phosphatase. A total of 749 and 779 amino acid residues are observed for isoenzyme I and isoenzyme II, corresponding to protein molecular weights of 84 000 and 85 000, respectively. The molecular weights of both isoenzymes were approx. 92 000 by exclusion chromatography. The differences in the molecular weights of these isoenzymes as determined by gel exclusion chromatography, SDS polyacrylamide gel electro-

TABLE III

AMINO ACID COMPOSITION OF ACID PHOSPHATASE ISOENZYMES

Except where otherwise indicated, integral residue values were calculated by least squares minimization of the results of 24-, 48- and 72-h hydrolysis reactions and rounding to the nearest integral value.

Amino acid	Rat liver isoenzyme I [a]	Rat liver isoenzyme I	Rat liver isoenzyme II	Human liver	Canine prostatic	Human prostatic [b]
Aspartic acid	94	70	82	64	51	54
Threonine [c]	66	52	73	57	63	50
Serine [c]	80	62	63	72	51	54
Glutamic acid	101	80	76	90	110	100
Proline	40	46	56	59	65	50
Glycine	96	47	51	53	43	42
Alanine	143	58	48	47	28	27
Valine	58	59	50	49	34	34
Methionine	8	17	16	13	22	20
Isoleucine	54	32	33	21	39	27
Leucine	67	73	80	83	89	93
Tyrosine [c]	32	33	24	29	46	43
Phenylalanine	34	32	26	32	36	32
Histidine	1	17	18	21	27	26
Lysine	65	44	45	35	47	45
Arginine	31	27	25	36	27	33
Cysteine	2	[e]	13	12		16
Tryptophan	4	[f]	[f]	[f]	[f]	[f]
Number of residues	976	749	779	773	778	764
Mol. wt. [d]	101 760	83 866	84 948	86 539	89 157	89 000

[a] As reported in ref. 5.

[b] As reported in ref. 7.

[c] Corrected for destruction by extrapolation to zero time.

[d] These figures represent the molecular weight due to amino acid residues; the contributions due to the carbohydrate portions of these glycoproteins are not included.

[e] A small but reproducible cysteine peak was observed but could not be accurately integrated at the sample sizes employed; a value of 1—2 residues seems likely.

[f] Not determined.

phoresis and by amino acid analysis are due to the presence of carbohydrate residues on these proteins. Table IV presents carbohydrate analysis data for canine prostatic acid phosphatase. It contains 4.0% carbohydrate by weight, a value which is half that found for human prostatic acid phosphatase [33]. Like the human prostatic enzyme it appears to contain a residue of fucose but the most abundant residues are glucosamine and mannose. We find that an isoenzyme [10] of human liver acid phosphatase which we have purified to homogeneity (unpublished data) is also a glycoprotein containing approximately 4% carbohydrate in the form of 5 residues of *N*-acetylglucosamine and 16 residues of mannose per $9 \cdot 10^4$ g of protein. In contrast to the results obtained for the prostatic enzyme [33] we find no *N*-acetylneuraminic acid, galactose or fucose in the human liver acid phosphatase. Although the carbohydrate contents of the rat liver acid phosphatase isoenzymes have not been quantitatively determined, it is certain that they too are glycoproteins since they are also retained on concanavalin-A-Sepharose and are eluted upon application of a low concentration of mannose. Additionally, the early (24 h) amino acid hydrolyzates of both isoenzymes showed the presence of significant amounts of glucosamine. Interestingly, isoenzyme II also showed a significant galactosamine content, indicating that these two isoenzymes may differ in the complexity of their carbohydrate residues. Since high estimates are obtained for the molecular weights of glycoproteins when determinations are made using SDS-gel electrophoresis on 7.5% gels [34], the observed molecular weight data are consistent with the results of the amino acid analyses (Table III).

Isoenzymes I and II of rat liver acid phosphatase, although generally similar in amino acid composition to each other and to the human liver and human and canine prostatic enzymes (Table III), may differ from one another when certain residues such as threonine are compared. However, the lysine, arginine and histidine contents of the two rat liver isoenzymes are effectively identical. It is probably of structural significance that the glycine and proline contents of all 5 isoenzymes (Table III) are relatively similar.

The number of histidine residues in rat liver acid phosphatase (Table III) is similar to the number of histidine residues in human liver and human prostatic acid phosphatases [7] and is much higher than reported by earlier workers [5]. Isoenzyme I of rat liver acid phosphatase had been previously reported [5] to contain only one histidine residue but we find 17 and 18 residues for isoenzyme I and isoenzyme II, respectively. A value of 21 histidine residues is ob-

TABLE IV

CARBOHYDRATE ANALYSIS OF CANINE PROSTATIC ACID PHOSPHATASE

Carbohydrate residue	Weight content (g/mol) *	Molar content (mol/mol) *	
		Mean	Integral value
Fucose	176	1.07	1
Galactose	333	1.85	2
Mannose	639	3.55	3—4
N-Acetylglucosamine	2454	12.64	12—13

* Given per mol protein as calculated from the amino acid composition.

served for the human liver enzyme (Table III) while a value of 26 was reported for human prostatic acid phosphatase [7]. The presence of more than one histidine residue in these dimeric enzymes is of importance because it has been established that an active site histidine residue is stoichiometrically phosphorylated in the course of hydrolysis reactions catalyzed by an acid phosphatase [30]. The essential nature of two histidine residues per (dimeric) enzyme molecule has been quantitatively established in covalent inhibition reactions of human prostatic acid phosphatase [31]. An essential active site histidine has also been implicated in the action of other acid phosphatases [5,28,35—37]. Although burst titration experiments were reported to establish the presence of one active site in rat liver acid phosphatase [5] they were conducted under conditions where $[S] << K_m$. As we have pointed out in the case of similar experiments on human prostatic [28] and wheat germ acid phosphatases [30], only when $[S] >> K_m$ does the burst stoichiometry approach the active site concentration.

Thus, the molecular weights, subunit components and amino acid compositions of rat liver acid phosphatase isoenzymes I and II are similar to those seen for human prostatic, canine prostatic and human liver acid phosphatases. We expect all of them to be structurally homologous and, in a gross structural sense, we also expect other acid phosphatase isoenzymes of molecular weight $90—110 \cdot 10^3$ (at least those from animal sources) to be dimeric proteins. In this sense, at least, the acid phosphatases resemble the metalloenzyme alkaline phosphatase, but the structural similarities between the two classes of phosphatases are probably much more extensive. Further studies on the comparative biochemistry and mechanism of action of acid phosphatases are in progress.

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