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THE HETEROGENEITY OF PROSTATIC ACID PHOSPHATASE

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SUMMARY

1. The heterogeneity of human prostatic acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) was investigated.

2. The enzyme was homogeneous with respect to molecular weight, but could be separated into two peaks on DEAE-cellulose. These two peaks were further purified by gel filtration, and their catalytic properties, including pH optima, substrate and inhibitor specificities and kinetic constants, were found to be identical.

3. The preparative separation on DEAE-cellulose was correlated with the resolution of the enzyme into about 20 bands on starch-gel electrophoresis. All bands were inhibited by L(+)-tartrate.

4. Experiments in which the enzyme was incubated with purified neuraminidase indicated that the electrophoretic heterogeneity of prostatic acid phosphatase was due to the attachment of different numbers of sialic acid residues to a single enzyme protein.

INTRODUCTION

At least four forms of prostatic acid phosphatase have been distinguished by immunological methods[1], at least two by ion-exchange chromatography[2,3], and about thirteen by starch-gel electrophoresis[4–6]. SUR, MOSS AND KING[4] and VERNON *et al.*[3] have suggested that the distinct forms of the enzyme differ only in their charge properties, but LUNDIN AND ALLISON[6] found differences in their inhibition by L(+)-tartrate. The latter authors proposed that, by analogy with lactate dehydrogenase, the banded electrophoretic pattern of acid phosphatase was due to random recombination of different polypeptide chains.

The present work was undertaken to correlate the separations achieved by preparative and electrophoretic methods, to investigate the enzyme's electrophoretic heterogeneity, and relate this to possible differences in catalytic properties.

It was found that the differences in the separations obtained by ion-exchange chromatography and electrophoresis were due solely to the superior resolving power of the latter technique, particularly when discontinuous buffer systems were used. The banded electrophoretic pattern was interpreted as a single enzyme protein

carrying increasing numbers of sialic acid residues, and the bands were found to have identical catalytic properties.

MATERIALS AND METHODS

Materials

The sodium salts of *p*-nitrophenyl phosphate and naphthyl 1-phosphate were obtained from British Drug Houses Ltd. All other substrates, crystalline lysozyme, hyaluronidase, bovine albumin and chromatographically purified neuraminidase (Type V from *Clostridium perfringens*) were obtained from Sigma, London.

Chromatography

Cellulose phosphate (Whatman, P-11) and DEAE-cellulose (Whatman, DE-32) were used in 2.5 cm × 90 cm glass columns. Sample enzyme solutions were predialysed against the buffer used to equilibrate the adsorbents. Sephadex G-150 and G-200 (Pharmacia, London) were used in glass or Perspex columns equipped for upward flow. All chromatographic runs were carried out at 4°. Other details of individual experiments are described in EXPERIMENTAL and legends.

When necessary, column effluents were concentrated by overnight dialysis against Carbowax (polyethylene glycol, mol. wt. > 20 000, Union Carbide), followed by brief dialysis against an appropriate dilute buffer. Recovery was almost quantitative.

Measurement of enzyme activity

Acid phosphatase activity of column effluents was measured by adding 1 ml 4 mM disodium *p*-nitrophenyl phosphate at 37° to a small volume of enzyme solution in 1 ml 0.1 M citrate buffer (pH 6.0) at 37°. After incubation at 37° for 5 min, the reaction was stopped by the addition of 1 ml 0.4 M NaOH, and the absorbance of *p*-nitrophenol measured at 400 mμ. 10 μg of bovine serum albumin was added to the incubation mixture when highly-purified acid phosphatase was assayed.

When other substrates were used, the release of P_i was measured by the method of BAGINSKI, FOA AND ZAK[7], which is unaffected by spontaneous hydrolysis of labile substrates after termination of the enzymic reaction. The incubation mixture consisted of 0.5 ml of 1.5 M acetic acid–NaOH buffer (pH 5.0) adjusted to $I = 1.5$ with NaCl, a small volume of enzyme solution (diluted, when highly purified, with 0.01% albumin), and 0.5 ml of 4 mM substrate added to start the reaction. The incubation time was 5 min. Recovery of a standard amount of P_i was constant from pH 2.5 to 7.5, and acid phosphatase activity was proportional to time of incubation and enzyme concentration over a 5-min incubation period.

Enzyme activity was expressed as μmoles *p*-nitrophenol or P_i released per min per ml enzyme solution, and an $A_{280\ m\mu} = 1.0$ was taken as approximately equivalent to 1 mg protein per ml.

Buffers for pH optimum experiments

Most buffers giving pH values in the range 3–7 include components which either inhibit acid phosphatase[8] or interfere with the subsequent determination of P_i (ref. 9). Citrate is the buffer of choice when measuring the release of *p*-nitrophenol,

but acetate must be substituted when measuring the release of P_i from substrates which are not chromogenic. 2 M or 1 M solution of free citric or acetic acid was adjusted to a range of pH values with 2 M NaOH, and made up to a fixed final volume. Acetate buffers of constant ionic strength ($I = 1.5$) were made by calculating the degree of dissociation of acetic acid at each pH value, and adding the appropriate concentration of NaCl to each buffer. pH electrodes were found to contaminate buffer solutions with an enzyme inhibitor, probably Hg^{2+}; acid phosphatase is affected by as little as 0.1 μM of this ion.

Starch-gel electrophoresis

Up to 12 parallel samples could be compared in a horizontal gel tray (30 cm long, 16 cm wide) and filled to a depth of 8 mm by 400 ml of 10% gel. This wide gel tended to shrink and crack at the anode end during long runs, due to the abrupt discontinuity of buffer concentration between the gel and the electrode vessels. The best compromise between gel stability and resolution was achieved in a system using different concentrations of the same pH 5.0 citrate buffer; 0.5 M and 0.1 M in the cathode and anode vessels, respectively, and 5 mM in the gel. An overall potential difference of 200 V, giving a current of about 35 mA, was applied for 20 h, the separatation being carried out in a cold room at 4°. Gels were stained for acid phosphatase by the method of ESTBORN[10].

The lower pH of the separation resulted in slower runs than those of SUR, MOSS AND KING[4], but the resolution was closely comparable. Discontinuous acetate buffers, and the discontinuous system of HOPKINSON, SPENCER AND HARRIS[11], gave inferior resolution.

EXPERIMENTAL

Enzyme extraction

Fresh autopsy specimens of normal human prostate were stripped and cut into small pieces, and homogenised at 4° in a Waring blendor for a total of 20 sec, in 4 vol. of 'cold' 0.01 M citrate buffer (pH 6.0). The supernatant was decanted, filtered at 4°, and stored at −20°.

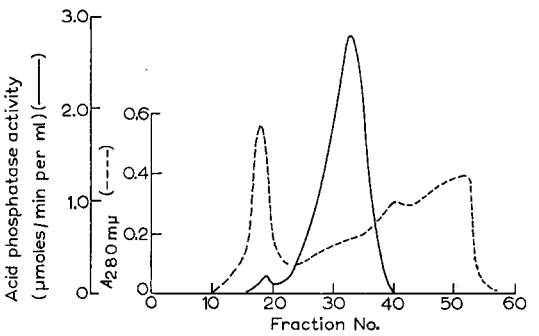

Fig. 1. Gel filtration on Sephadex G-200. 10 ml of uncentrifuged prostatic homogenate was applied to a 3 cm × 90 cm column of Sephadex G-200, and the column eluted upwards with 0.01 M citrate buffer (pH 6) containing 0.1 M NaCl. The flow rate was about 7 ml/cm² per h, and 16-ml fractions were collected. ———, acid phosphatase activity; — — —, $A_{280\ m\mu}$.

Molecular weight homogeneity

Fig. 1 shows the distribution of acid phosphatase activity after gel filtration of the homogenate on Sephadex G-200. A small proportion of activity was eluted with the void volume; this enzyme peak did not appear if the sample was first centrifuged at 100 000 × *g* for 30 min, and it was attributed to particle-bound enzyme. The second peak, representing 90–100% of the applied activity, was always homogeneous and the extreme edges of the peak, after concentration and re-filtration, were eluted in identical volumes: the entire peak therefore consisted of enzyme species differing in molecular weight by less than 5%. Calibration of the column by filtration of albumin and its dimer gave an estimated mol. wt. of 105 000 for the enzyme, which compares well with previous estimates[2,3]. The starch-gel electrophoretic patterns of both the major and 'particle-bound' peaks were identical with that of the homogenate.

Purification of two enzyme peaks from DEAE-cellulose chromatography

Preliminary experiments showed that the acid phosphatase in the prostatic homogenate could be separated into two peaks on DEAE-cellulose. Since, however,

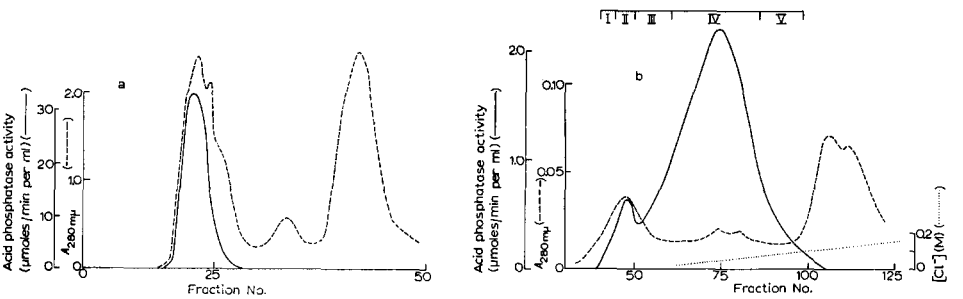

Fig. 2a. Chromatography on cellulose phosphate. 50 ml prostatic homogenate was applied to a 2.5 cm × 90 cm column of cellulose phosphate, equilibrated with 0.01 M citrate buffer (pH 6). The same buffer was used to elute the columns at about 50 ml/h. After all unadsorbed components had been eluted, the same column could be used again without re-equilibration. 15-ml fractions were collected. ———, acid phosphatase activity; — — —, $A_{280\,m\mu}$.

Fig. 2b. Chromatography on DEAE-cellulose. 10 ml concentrated effluent from cellulose phosphate was applied to a 2.5 cm × 90 cm column of DEAE-cellulose, equilibrated with 0.01 M Tris–citrate buffer (pH 7.5). A linear gradient of NaCl, from 0 to 0.2 M over 2 l, in the same buffer was used to elute the column at about 50 ml/h. 15-ml fractions were collected. Pools I–V were concentrated to small volume for starch-gel electrophoresis (Fig. 2c), and Fractions II and IV purified further by gel filtration. ———, acid phosphatase activity; — — —, $A_{280\,m\mu}$; . . . . . , $[Cl^-]$ in effluent.

the exchanger had a very low capacity under the conditions for maximum resolution of the two enzyme peaks, the critical DEAE-cellulose separation was preceded by a crude fractionation of the homogenate on cellulose phosphate. The two peaks separated on DEAE-cellulose were further purified by gel filtration.

The prostatic homogenate was first passed through cellulose phosphate, equilibrated with 0.01 M citrate (pH 6.0). Acid phosphatase emerged as a single peak, and was recovered in 90% yield (Fig. 2a).

Fractions constituting this peak were pooled, concentrated and chromato-

graphed on DEAE-cellulose in 0.01 M Tris–citrate (pH 7.5) under a shallow salt gradient (Fig. 2b). Total recovery of applied enzyme activity was 75–85%. The proportion of this found in the first peak varied with each prostatic homogenate, ranging from 20 to 40%. When fractions across the elution profile were separately pooled, concentrated, and subjected to starch-gel electrophoresis, it was found that all (except possibly the very fastest) bands present in the homogenate were recovered in one or more of the eluted fractions (Fig. 2c).

Fractions II and IV were then separately passed through the same 2.5 cm × 30 cm column of Sephadex G-150, emerging in identical elution volumes. The yield was more than 90%. The most active fractions from gel filtration, called 'purified Fractions II and IV', represented the two main peaks from DEAE-cellulose chromatography, and the slow and fast regions, respectively, of the electrophoretic pattern. Purified Fractions II and IV had specific activities 88 and 160 units per $A_{280\ m\mu}$, respectively, constituting 50- and 100-fold purifications from the prostatic homogenate.

Before comparing the properties of purified Fractions II and IV, tests were carried out to ensure that they were free from contamination by other phosphatases.

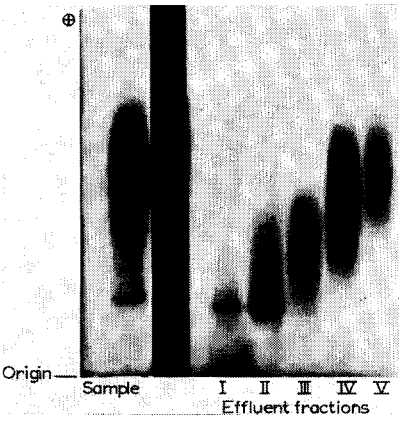

Fig. 2c. Starch-gel electrophoresis of DEAE-cellulose fractions. Concentrated pooled fractions from DEAE-cellulose chromatography (Fig. 2b) were subjected to starch-gel electrophoresis under the conditions described in METHODS. Fractions II and IV, selected for further purification and comparison, were representative of the 'slow' and 'fast' regions, respectively, of the electrophoretic pattern of the whole homogenate.

They had negligible glucose-6-phosphatase or pyrophosphatase activity (acetate, pH 5) or alkaline phosphatase activity (carbonate–bicarbonate, pH 10). The enzymes hydrolysed adenosine 5-monophosphate, but this activity could not be attributed to contamination by specific 5'-nucleotidase and appeared to be a property of prostatic acid phosphatase, since the profiles of activity towards *p*-nitrophenyl phosphate and adenosine 5'-monophosphate across effluents from Sephadex and DEAE-cellulose were identical. Furthermore, the hydrolysis of neither substrate was activated[12] by 10 mM Mn^{2+}, while the hydrolysis of both substrates was inhibited[12] about 50% by 10 mM Ni^{2+}, and more than 90% by 5 mM L(+)-tartrate. The hydrolysis of nucleotides was not confined to the 5'-esters.

Comparison of catalytic properties of the two enzyme peaks

During the search for a common buffer system in which the hydrolysis of all substrates could be measured over a wide pH range, it was found that prostatic acid phosphatase activity was highly dependent on the ionic strength of the assay buffer. In summary, the effect of increasing I from about 0.05 to 0.5 was to diminish activity at pH values lower than 4 and greater than 6. The effects of ionic strength were therefore minimised by using a concentrated buffer (final acetate concentration in the incubation mixture, 0.5 M) and adding NaCl to all buffers below pH 6 to give a final total I of 0.75 in the incubation mixture. These constant ionic

TABLE I

SUBSTRATE SPECIFICITY OF PURIFIED FRACTIONS OF PROSTATIC ACID PHOSPHATASE AT pH 5

(*a*) The rates of hydrolysis of several substrates by the purified Fractions II and IV (Figs. 2b and c) were compared with the rate of hydrolysis of the reference substrate (*p*-nitrophenyl phosphate = 100%). (*b*) The most rapidly hydrolysed substrates were also incubated with and without 5 mM L(+)-tartrate, and the percentage inhibition effected by tartrate was calculated. (*c*) Each enzyme fraction was made to hydrolyse 2 mM *p*-nitrophenyl phosphate, with and without the addition of other substrates, also 2 mM. The release of P_i or *p*-nitrophenol was measured as described in the METHODS section.

Substrate (2 mM)	*(a) Relative rate of hydrolysis*		*(b) % Inhibition by 5 mM L(+)-tartrate*		*(c) % Inhibition of p-nitrophenyl phosphate hydrolysis*	
	Fraction II	*Fraction IV*	*Fraction II*	*Fraction IV*	*Fraction II*	*Fraction IV*
Monophosphoric esters						
p-Nitrophenyl phosphate	100	100	91	91		
Naphthyl 1-phosphate	145	135	70	76	75	74
Naphthyl 2-phosphate	135	126	72	77	72	72
Glucose 1-phosphate	2	5			0	0
Glucose 6-phosphate	1	2			0	0
α-Glyceryl phosphate	21	30		98	14	4
β-Glyceryl phosphate	60	83		99	22	9
Adenosine 2′-monophosphate	60	66	95	97	31	36
Adenosine 3′-monophosphate	107	122	95	95	45	45
Adenosine 5′-monophosphate	35	48	95	97	34	39
Uridine 5′-monophosphate	27	36	98	99	24	12
Serine phosphate	0	0			9	9
Threonine phosphate	0	0				
Pyridoxal phosphate	80	76		97		
Phosphoenolpyruvic acid	1	2				
Pyrophosphates						
Inorganic pyrophosphate	1	2				
Adenosine 5′-diphosphate	2	2			21	20
Nicotinamide adenine dinucleotide	0	3			12	13
Adenosine 5′-triphosphate	0	1			26	12
Diesters, triesters						
Bis-*p*-nitrophenyl phosphate	2	3			0	0
Tris-*p*-nitrophenyl phosphate	0	0			0	0
Diphosphates						
Fructose 1,6-diphosphate	2	3			3	0

strength buffers were used in the determination of pH optima, and the pH 5 buffer was used in all other kinetic experiments on the purified enzyme peaks.

The pH dependence of the hydrolysis of three substrates by purified acid phosphatase is shown in Fig. 3. The enzyme solution used in these experiments was purified Fraction II, but purified Fraction IV gave identical curves. The same curves for the hydrolysis of *p*-nitrophenyl phosphate were obtained when the release of *p*-nitrophenol and P_i were measured simultaneously. It is emphasised that the pH optima and other kinetic data presented here are valid only for the particular buffer used. The concentrated acetate buffer finally selected had the advantage of closely controlling pH in the presence of other assay components, and of sharpening the optimum curves, which are very flat in dilute buffers. Similar pH curves for the

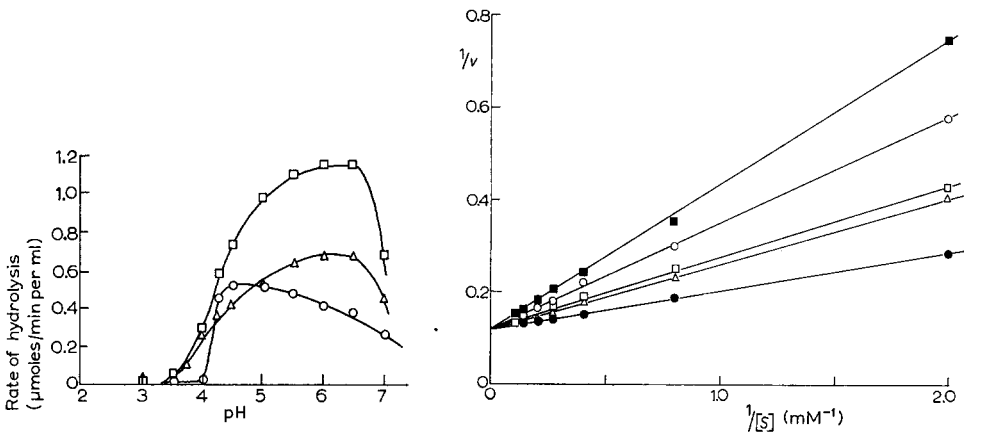

Fig. 3. pH optima. The rates of hydrolysis of three substrates by purified Fraction II were measured in constant-ionic strength (I = 0.75) acetate–NaCl buffers under the conditions described in the METHODS section (P_i method). Different dilutions of enzyme were used for each substrate. The same curves were obtained when purified Fraction IV was used as enzyme. Substrates (all 2 mM); □, adenosine 5′-monophosphate; △, β-glycerophosphate; ○, *p*-nitrophenyl phosphate.

Fig. 4. Competitive inhibition of *p*-nitrophenyl phosphate hydrolysis. The rate of hydrolysis (v) of *p*-nitrophenyl phosphate, at pH 5 in acetate–NaCl buffer (I = 0.75), was measured at several substrate concentrations [S], with and without the addition of inhibitors. Double-reciprocal plots fitted straight lines converging on the ordinate axis. A few experimental points near the convergence have been omitted in the interest of clarity. ●, no inhibitor; ■, molybdate, 0.01 mM; ○, meta-vanadate, 0.01 mM; □, arsenate, 10 mM; △, fluoride, 1 mM.

hydrolysis of *p*-nitrophenyl phosphate were found using either the chosen acetate buffer or 0.5 M citrate buffer (*cf.* ref. 13).

The specificity of acid phosphatase for a range of phosphomonoesters and related compounds was determined at pH 5. Table I shows that there were no marked differences in the relative rates of hydrolysis of substrates by the two purified fractions. They both had very broad specificity for phosphomonoesters, hydrolysing the esters of aliphatic, aromatic and heterocyclic alcohols at comparable rates. Sugar phosphates were scarcely hydrolysed, however, and the virtual absence of activity towards the esters of serine, threonine and enolpyruvic acid may indicate inhibition

by neighbouring carboxyl groups. Diphosphates, multiple esters and pyrophosphates were virtually unattacked, the latter even in the presence of Mg^{2+}.

In consideration of the phosphotransferase capacity of prostatic acid phosphatase[14], the release of *p*-nitrophenol, rather than the release of P_i, was measured in the mixed-substrate experiments. None of the unhydrolysed phosphates was a very potent inhibitor of the enzyme's activity towards *p*-nitrophenyl phosphate, and all the rapidly hydrolysed monoesters appeared to compete at the same catalytic centre. The activity of both purified enzyme fractions towards all substrates was strongly inhibited by 5 mM L-(+)-tartrate, the less complete inhibition in the case of the naphthyl esters being a reflection of the competitive type of inhibition[15].

The enzyme could be inhibited by many organic and inorganic ions, *e.g.*, nitrate, bromide, phosphate and Tris, under different conditions of pH, ionic strength and inhibitor concentration. Strong competitive inhibition by molybdate, meta-vanadate, arsenate and fluoride provided quantitative comparisons of the two purified enzyme fractions. From double-reciprocal plots of substrate concentration and acid phosphatase activity in the presence and absence of each inhibitor, the K_m and four K_i values for each enzyme were calculated. The graphical evidence of purely competitive inhibition is presented in Fig. 4, and the kinetic constants are compared in Table II.

TABLE II

KINETIC CONSTANTS FOR TWO PURIFIED FRACTIONS OF PROSTATIC ACID PHOSPHATASE AT pH 5

Constants were calculated from the reciprocal plots of Fig. 4 for Fraction II, and similar plots for Fraction IV of purified enzyme. Inhibitor was added to the enzyme and buffer less than 5 min before the addition of substrate, 2 mM *p*-nitrophenyl phosphate in each case. The release of *p*-nitrophenol was measured as described in the METHODS section.

Inhibitor	K_m		K_i	
	Purified enzyme Fraction II	*Fraction IV*	*Fraction II*	*Fraction IV*
None	0.74 mM	0.69 mM		
Molybdate, 0.01 mM			3.3 μM	3.1 μM
Meta-vanadate, 0.01 mM			5.7 μM	5.8 μM
Fluoride, 1.0 mM			1.4 mM	1.5 mM
Arsenate, 10 mM			11 mM	8 mM

Electrophoretic heterogeneity

Fig. 5 shows the electrophoretic pattern of a homogenate of a single prostate, run under the conditions described in the METHODS section. All electrophoretic bands were almost totally inhibited by the addition of 5 mM L(+)-tartrate to the staining solution. At least 20 regularly-spaced bands were visibly resolved in the uninhibited gel, and these have been numbered to simplify discussion. After electrophoresis in any buffer system of pH about 6, two main zones of staining were seen, corresponding to band 3 and the region of bands 10–16, but these areas were resolved into discrete bands only by the narrowly-defined discontinuous citrate buffer system. All ten homogenates of individual prostates examined gave similar patterns, although the area of maximum intensity in the fast region varied, bands 1 and 2 were often very faint, and some homogenates lacked the very fastest bands. Ageing of the

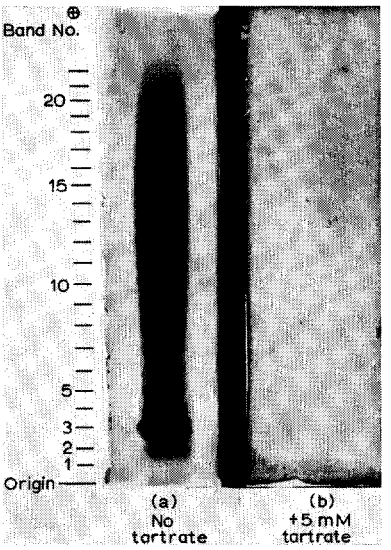

Fig. 5. Inhibition of all starch-gel zones of acid phosphatase by 5 mM L (+)-tartrate. A single sample of prostatic homogenate was subjected to starch-gel electrophoresis under the conditions described in the METHODS section. After slicing the gel, the halves were covered with (a) 5 mM naphthyl 1-phosphate, and (b) 5 mM naphthyl 1-phosphate + 5 mM L(+)- tartrate. The released 1-naphthol was coupled after 5 min with a solution of tetrazotised *o*-dianisidine (Fast Blue B), In the original gel (b), only bands 3 and 13–18 were faintly visible. In this particular homogenate, band 1 was too faint to show in the photograph.

whole prostate or homogenate, autolysis, and incubation at 37° seemed also to diminish the fastest components, but it was impossible to tell whether this activity was redistributed among the slower zones. The spacing of the bands was identical in all homogenates, and matching bands could be precisely identified; mixtures of homogenates therefore gave patterns indistinguishable from those of single homogenates. This alignment was not due to the passage of a 'front' of any buffer component, since identical homogenate samples inserted in staggered slots in the same gel gave staggered rather than parallel patterns. The banded pattern was not due to non-specific binding by contaminating proteins, since highly purified fractions were similarly resolved.

Effect of neuraminidase on the electrophoretic mobility of acid phosphatase

Attempts were made to digest the prostatic homogenate with each of three enzymes, hyaluronidase, lysozyme and neuraminidase, known to hydrolyse glycosidic bonds between acidic sugar moieties. The homogenate was incubated at 37° for increasing periods with each of the enzymes in 0.1 M acetate or citrate buffer (pH 5.0). After incubation for 48 h, hyaluronidase and lysozyme had had no effect on acid phosphatase, but neuraminidase had produced a redistribution of the faster electrophoretic components among the slower bands. This experiment was repeated using a fresh solution of the purest neuraminidase available, and the possibility that the decrease in mobility might be due to non-specific binding by neuraminidase was eliminated by showing that the effect was dependent on both incubation time and neuraminidase concentration (Fig. 6); when the neuraminidase preparation was

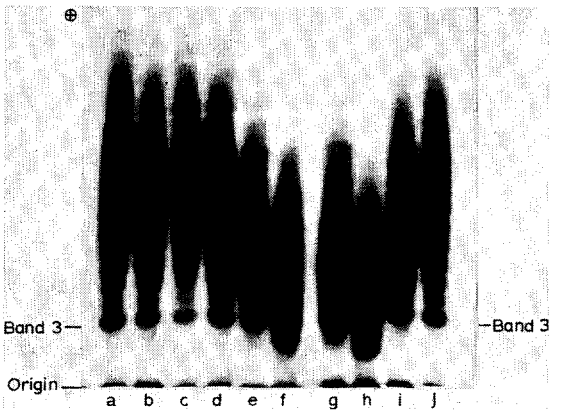

Fig. 6. Alteration of the electrophoretic mobility of prostatic acid phosphatase by digestion with neuraminidase. 1 ml prostatic homogenate was incubated at 37°, in 0.1 M citrate buffer (pH 5) with increasing amounts of chromatographically purified neuraminidase for increasing periods. Digestion mixtures were rapidly frozen after removal from the 37° water bath, then thawed simultaneously and run in parallel on a starch gel as described in the METHODS section. (a) Control, no neuraminidase, no incubation; (b) control, no neuraminidase, incubated 24 h; (c) control, 10 μg neuraminidase, no incubation; (d) digest, 10 μg neuraminidase, incubated 1 h; (e) digest, 10 μg neuraminidase, incubated 5 h; (f) digest, 10 μg neuraminidase, incubated 24 h; (g) control, 100 μg neuraminidase, no incubation; (h) digest, 100 μg neuraminidase, incubated 24 h; (i) digest, 1 μg neuraminidase, incubated 24 h; (j) digest, 0.1 μg neuraminidase, incubated 24 h. Although sample (g) was not incubated at 37°, the acid phosphatase was in solution with this very high concentration of neuraminidase for a few minutes at 5° during sample application, and for an unknown period during the electrophoretic run.

boiled for 5 min, without visible precipitation, before addition to acid phosphatase, no alteration of the electrophoretic pattern was seen.

Some idea of the order in which the electrophoretic bands were attacked was gained from comparisons of the starch-gel patterns of samples withdrawn at different intervals during the incubation of neuraminidase with the prostatic homogenate and with fractions eluted from DEAE-cellulose. The fastest bands were attacked first, until the bulk of the enzyme activity was compressed into bands 3–10, after which these bands were much more slowly digested, and bands 1 and 2 increased in prominence. Even when bands 1–5 had become about equally intense, no trace of a slower band was observed. The initial mobility of an electrophoretic band did not appear to affect its final mobility after prolonged digestion. The slowest band observed (band 1) almost certainly represents the end product of digestion, since dialysis of the digest during incubation did not increase the extent of the digestion, and since the neuraminidase in the final digest was still active on freshly added acid phosphatase.

DISCUSSION

Catalytic properties of purified acid phosphatase

Very highly purified prostatic acid phosphatase has been prepared by several groups of workers[2,3,13,16], although often in a rather unstable state, and without information about electrophoretic homogeneity of the final product and the fractions of low specific activity discarded during purification. The purification carried out in

the present work, similar in many ways to that of VERNON *et al.*[3], was aimed rather at preserving as many as possible of the acid phosphatase components of the initial prostatic homogenate, while freeing the enzyme from all contaminating phosphatases which might lead to ambiguity in interpreting kinetic data. The purified Fractions II and IV were judged to be free from glucose 6-phosphatase, alkaline phosphatase, pyrophosphatase and 5-nucleotidase.

VERNON *et al.*[3] could find no difference in the kinetic properties of two enzyme peaks separated on DEAE-cellulose. The representative purified Fractions II and IV compared in the present work did not differ significantly in any of the properties examined in Tables I and II, supporting the view that the many electrophoretic forms of acid phosphatase result from superficial variations in the enzyme's structure which do not affect its catalytic properties. For most purposes, therefore, the prostatic acid phosphatase may be regarded as a single enzyme. In particular, it was confirmed that all electrophoretic bands were inhibited by L(+)-tartrate[4]. This is important because of the widespread use of this inhibitor to differentiate prostatic acid phosphatase from other phosphatases in human serum. LUNDIN AND ALLISON's claim[6] that one electrophoretic band is selectively inhibited may have arisen from their preincubating the gel with tartrate, rather than adding this competitive inhibitor to the substrate solution.

Source of the enzyme's charge heterogeneity

Experiments in which prostatic homogenate and purified fractions were digested with neuraminidase of the highest purity showed that the enzyme could undergo progressive removal of acidic groups (although the scale of the experiments was too small to permit the unequivocal identification of sialic acid in the digest). Considering the large number of bands, and the minimal differences in their molecular weights shown by gel filtration, it seems probable that the electrophoretic heterogeneity of the enzyme arises from a single enzyme protein bearing a variable number of acidic residues. VERNON *et al.*[3] reported that the enzyme peaks separated on DEAE-cellulose had sedimentation coefficients differing by about 2.5%. This difference could be accounted for by a difference of about 10 sialic acid residues. The zones of maximum intensity in starch-gel patterns, corresponding to the two peaks on DEAE-cellulose (Figs. 2b and c) are separated by about 10 bands.

The mild conditions under which the enzyme was extracted, and the difficulty in altering electrophoretic mobility by autolysis, suggest that the attachment of acidic groups occurs intracellularly and is not an artifact of the extraction procedure.

The sequence in which the electrophoretic bands were eliminated during digestion with neuraminidase indicates that the phosphatase protein carries only one or a few chains of repeating sialic acid units, rather than a large number of singly-attached units, and that the glycosidic bonds are more resistant to hydrolysis near the protein. Band 1 may not, therefore, consist of protein completely stripped of sialic acid.

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