Purification and Some Properties of Tartrate-Sensitive Acid Phosphatase from Rabbit Kidney Cortex*

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Two forms of tartrate-sensitive acid phosphatases (EC 3.1.3.2) were purified from rabbit kidney cortex by a multiple-column-chromatography method. The basic form constituted 90% of the enzyme and migrated as a single band of protein on polyacrylamide-gel electrophoresis. The proteins contaminating the acidic form did not exceed 5% of the total protein. The specific activity towards *p*-nitrophenyl phosphate was 12 $\mu\text{mol}/\text{min}$ per mg for the basic form and 0.7 $\mu\text{mol}/\text{min}$ per mg for the acidic form. The basic form of the enzyme differs from the acidic form in its heat-stability, K_m values, inhibition rates by tartrate and fluoride and substrate specificities. Relative to *p*-nitrophenyl phosphate hydrolysis rate, the acidic form hydrolysed a variety of physiological monophosphate esters, whereas the basic form hydrolysed only CMP and phosphoenolpyruvate. Bacterial neuraminidases had no effect on the activity and mobility of the acidic form on polyacrylamide-gel electrophoresis. Both forms have the same molecular weight (101000 ± 4000) and are probably composed of two identical subunits. The question whether the two forms of the enzyme are different proteins or whether one is a modified form of the other is discussed.

Acid phosphatases [orthophosphoric-monoester phosphohydrolase (acid optimum)], the group of enzymes catalysing the hydrolysis of phosphoesters in an acidic medium, are widely distributed in Nature and often occur in multiple forms in various tissues. The multiple forms of acid phosphatases have been separated and partially purified from various tissues (Fenton & Richardson, 1967; DiPietro & Zengerle, 1967; Heinrikson, 1969; Filburn, 1973; Wasyl, 1975). Rehkop & Van Etten (1975) have reported the occurrence and partial purification of three forms of acid phosphatase from human liver. These forms of acid phosphatase differ from each other in their molecular weight (Heinrikson, 1969; Rehkop & Van Etten, 1975), substrate specificity (Igarashi & Hollander, 1968; DiPietro, 1968) and inhibition by several reagents, e.g. sodium tartrate, sodium fluoride and formaldehyde (Filburn, 1973). The origin as well as the functional and structural relations of these forms are obscure. *N*-Acetylneuraminic acid attachment to the enzyme molecule is known to be partly responsible for the multiplicity of a given-molecular-weight acid phosphatase in human prostate (Smith & Whitby, 1968; Ostrowski *et al.*, 1970) and pig liver (Campbell *et al.*, 1973).

Abbreviation used: SDS, sodium dodecyl sulphate.

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Earlier work from this laboratory has indicated the presence of three forms of acid phosphatase in rabbit kidney cortex designated I, II and III in order of their elution from a Sephadex G-150 column (Helwig *et al.*, 1977a). These forms differ from each other in their stabilities, response to various inhibitors and distribution in renal glomeruli and tubules. Form I, the most stable one of kidney acid phosphatase, is strongly inhibited by sodium tartrate and fluoride (Helwig *et al.*, 1977a). The present paper describes the purification and properties of tartrate-sensitive acid phosphatase (form I) from rabbit kidney cortex.

Materials and Methods

Materials

p-Nitrophenyl phosphate (disodium salt) was purchased from E. Merck (Darmstadt, Germany). Methyl α -D-mannoside, Coomassie Brilliant Blue, α -naphthyl phosphate, glycerol 2-phosphate, D-fructose 1,6-bisphosphate, D-glucose 6-phosphate, 2,3-bisphosphoglyceric acid, phosphoenolpyruvate, D-ribose 5-phosphate, flavin mononucleotide, pyridoxal 5'-phosphate, adenosine 5'-monophosphate, adenosine 5'-triphosphate, guanosine 5'-monophosphate, uridine 5'-monophosphate, cytidine 5'-monophosphate, neuraminidase type VI (*Clostridium*

perfringens) and haemoglobin were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Sephadex G-150, SP (sulphopropyl)-Sephadex C-50 and concanavalin A-Sepharose were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). DEAE-cellulose DE-52 was purchased from Whatman (Reeve Angel, London). Acrylamide and *NN'*-methylenebisacrylamide were obtained from Eastman Organic Chemicals, Distillation Product Industries (Rochester, NY, U.S.A.). Horse myoglobin, chymotrypsinogen A, ovalbumin, bovine serum albumin and aldolase were purchased from Serva Feinbiochemica (Heidelberg, Germany). Catalase was obtained from Worthington Biochemical Corp. (Freehold, NJ, U.S.A.). Neuraminidase (*Vibrio cholerae*) was obtained from Calbiochem (San Diego, CA, U.S.A.).

Assay of acid phosphatases

The enzyme was assayed by measuring the production of *p*-nitrophenol from *p*-nitrophenyl phosphate. The reaction mixture consisted of 0.9 ml of 50 mM-sodium citrate buffer, pH 4.8, 0.1 ml of 55 mM-*p*-nitrophenyl phosphate in the same buffer and up to 100 µg of protein according to the degree of enzyme purification (*p*-nitrophenol absorption coefficient at 405 nm = 15 500 litres · mol⁻¹ · cm⁻¹) in a total volume of 1.1 ml. The reaction mixture was incubated for 0.5 h at 37°C and the reaction was stopped by the addition of 0.5 ml of 0.4 M-NaOH. The amount of *p*-nitrophenol formed was determined spectrophotometrically at 405 nm. One unit of enzyme was defined as the amount liberating 1 µmol of *p*-nitrophenol/min under the above conditions. The specific activity was expressed as units/mg of protein. Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

In the substrate-specificity study the *p*-nitrophenyl phosphate was replaced by 0.1 ml of 100 mM-substrate (Table 4). The tubes were incubated at 37°C for 1.5 h. The liberated P_i was measured by the method of Weil-Malherbe & Green (1951). Appropriate reagent and enzyme blanks were included for all substrates and enzyme samples. With phosphorylated compounds there was an inhibition of colour development (Filburn, 1973). This was corrected by measuring the degree of inhibition of colour yield with a standard amount of P_i. In every case, enzyme activity was measured in a range of concentrations, where proportionality between the reaction rate and the amount of protein was assured.

Preparation of the acidic and basic forms of tartrate-sensitive acid phosphatase

All operations were carried out at 4°C unless otherwise mentioned. Kidney cortices (140–160 g)

from 18 rabbits (Fauves de Bourgogne) were homogenized six times in 5 mM-Tris/HCl buffer, pH 7.4, containing 20 mM-NaCl in a VirTis apparatus for 2 min at intervals of 20 s (two kidney cortices in a total volume of 30 ml). The cortical homogenate, after freezing and thawing five times, was centrifuged at 105 000 g for 30 min in a Spinco model L ultracentrifuge with rotor R-30. The supernatant was retained and the pellet was washed twice in a total volume of 540 ml of 5 mM-Tris/HCl buffer, pH 7.4, containing 20 mM-NaCl and 2 mM-EDTA and centrifuged for 1 h as described above. The pellet was discarded, and the three supernatants were combined and their pH was adjusted to 4.8 by the addition of 0.5 M-sodium citrate buffer, pH 4.5. This solution was kept overnight and centrifuged at 5000 g for 1 h in a MSE model 6L centrifuge. The pellet was discarded and the supernatant was made 40% saturated by the addition of solid (NH₄)₂SO₄ with constant mechanical stirring. After 1 h the solution was centrifuged at 5000 g for 1 h in an MSE model Mistral 6L centrifuge. The supernatant was discarded and the precipitate was dissolved in about 350 ml of 0.1 M-Tris/HCl buffer, pH 7.4, containing 20 mM-NaCl and 2 mM-EDTA and dialysed against 8 litres of 5 mM-Tris/HCl buffer, pH 7.4, containing 20 mM-NaCl and 2 mM-EDTA for 18 h. The dialysed solution was centrifuged at 105 000 g for 30 min as described above. The pellet was discarded and the volume of the supernatant was adjusted to 400 ml. This preparation is called the 'final extract'.

Sephadex G-150 gel filtration

The final extract was applied in four portions to four different columns (5 cm × 80 cm) of Sephadex G-150 equilibrated with 5 mM-Tris/HCl buffer, pH 7.4, containing 20 mM-NaCl and 2 mM-EDTA. The columns were eluted in 15 ml fractions with the same buffer, at a flow rate of 1.35 ml/min (Fig. 1). The fractions that contained the first peak of enzyme activity were pooled and made 40% saturated by the addition of solid (NH₄)₂SO₄ with constant mechanical stirring. After 1 h the solution was centrifuged at 5000 g for 1 h in an MSE centrifuge. The supernatant was discarded and the pellet was dissolved in 100 ml of 5 mM-Tris/HCl buffer, pH 7.4, containing 20 mM-sodium chloride and 2 mM-EDTA. This solution was dialysed overnight against 1 litre of 10 mM-sodium acetate buffer, pH 5.0, containing 2 mM-EDTA, with two changes. The turbid dialysed solution was centrifuged at 5000 g for 1 h in an MSE centrifuge and the supernatant (80 ml) was subjected to SP-Sephadex chromatography.

SP-Sephadex C-50 chromatography

To the above supernatant (80 ml), with slow mechanical stirring, was added 250 ml of sedimented

Fig. 1. Elution profile of rabbit kidney-cortex acid phosphatases through a Sephadex G-150 column

Enzyme activity was extracted in the presence of 2mM-EDTA (●) or 2mM-dithiothreitol (○). The experimental conditions and the analysis of fractions for enzyme activity and proteins (----) are described in the Materials and Methods section.

Fig. 2. Elution profile of rabbit kidney-cortex tartrate-sensitive acid phosphatases (AP) through an SP-Sephadex C-50 column

The experimental conditions and the analysis of fractions for enzyme activity (●) and for proteins (----) are described in the Materials and Methods section. Proteins were tested on the pooled fractions from effluent and 1M-NaCl eluate, and of each fraction from 0.15M-NaCl eluate.

SP-Sephadex equilibrated in 10mM-sodium acetate buffer, pH 5.0, containing 2mM-EDTA. After 10 min this suspension was packed in a 2.5cm×60cm column which was washed with 500ml of 10mM-sodium acetate buffer, pH 5.0, containing 2mM-EDTA, at a flow rate of 0.4ml/min collected in 4ml fractions. The enzyme appeared as a sharp peak when the column was washed with the starting buffer containing 0.15M-NaCl (Fig. 2). The active fractions were pooled and dialysed against the starting buffer.

SP-Sephadex C-50 re-chromatography

The dialysed solution (40ml) was again applied to an SP-Sephadex column (2cm×18cm) equilibrated in 10mM-sodium citrate buffer, pH 5.0, containing

Fig. 3. Rechromatography of rabbit kidney-cortex tartrate-sensitive acid phosphatases through an SP-Sephadex C-50 column

The experimental conditions and the analysis of fractions for enzyme activity (●) and for proteins (----) are described in the Materials and Methods section. A-AP and B-AP: acidic and basic forms of the tartrate-sensitive acid phosphatase.

Fig. 4. Polyacrylamide-gel electrophoresis of the SP-Sephadex rechromatography fractions containing acid phosphatase activity

Electrophoresis was carried out in the system 1 and stained for acid phosphatase activity. Experimental conditions are described in the Materials and Methods section. The numbers on the gels correspond to SP-Sephadex fractions. A-AP and B-AP: acidic and basic forms of the tartrate-sensitive acid phosphatase.

2mM-EDTA. The column was washed with the same buffer in the form of 4ml fractions at a flow rate of 0.4ml/min. After 120ml of the starting buffer had passed through the column, the elution was continued with a pH gradient formed from 2×200ml of the starting buffer and 1×200ml of 10mM-Na₂HPO₄, pH 8.4. The enzyme activity was eluted as a sharp peak between fractions 58–74 (Fig. 3). Each of these fractions was subjected to polyacrylamide-gel electrophoresis in system 1 (see under 'Polyacrylamide-gel electrophoresis') and stained for acid phosphatase activity. Fractions 58–65 contained the fast-moving acid phosphatase (acidic form: acid phosphatase-A)

Fig. 5. Elution profiles of acidic and basic forms of tartrate-sensitive acid phosphatase from rabbit kidney cortex through a DEAE-cellulose DE-52 column

Experimental conditions for enzyme activity determination are described in the Materials and Methods section. The acidic form (a) is fixed on the column, whereas the basic form (b) is not adsorbed on the column. A-AP and B-AP: acidic and basic forms of the tartrate-sensitive acid phosphatase.

and fractions 66–73 contained slow-moving acid phosphatase (basic form: acid phosphatase-B) (Fig. 4). The fractions containing acidic and basic forms were pooled separately and dialysed overnight against 5 mM-Tris/HCl buffer, pH 7.4, containing 2 mM-EDTA, with two changes.

DEAE-cellulose DE-52 chromatography

The dialysed solutions of acid phosphatase-A (35 ml) and acid phosphatase-B (35 ml) were separately applied on DEAE-cellulose DE-52 columns (0.8 cm × 7 cm) equilibrated in 5 mM-Tris/HCl buffer, pH 7.4, containing 2 mM-EDTA. The columns were washed with the same buffer collected in 4 ml fractions at a flow rate of 0.4 ml/min. Acid phosphatase-B is not adsorbed on the column whereas

acid phosphatase-A was completely retained (Fig. 5). Acid phosphatase-A appeared as a sharp peak when the column was eluted with 100 mM-NaCl in the starting buffer. The active fractions of each enzyme were pooled and dialysed against 2 litres of 20 mM-Tris/HCl buffer, pH 7.4, containing 150 mM-NaCl, 1 mM-MnCl₂ and 1 mM-CaCl₂.

Concanavalin A-Sepharose chromatography

The two solutions of acid phosphatase-A (10 ml) and acid phosphatase-B (70 ml) were applied to two separate concanavalin A-Sepharose columns (0.8 cm × 7 cm) equilibrated in 20 mM-Tris/HCl buffer, pH 7.4, containing 150 mM-NaCl, 1 mM-MnCl₂ and 1 mM-CaCl₂. The elution was performed as described earlier (Helwig *et al.*, 1977b). The active fractions of acid phosphatases-A and -B from concanavalin A-Sepharose columns were pooled, concentrated and dialysed against 20 mM-Tris/HCl buffer, pH 7.4, containing 2 mM-EDTA. These solutions can be stored at 4°C for at least 6 weeks without loss of activity.

Polyacrylamide-gel electrophoresis

Three polyacrylamide-gel electrophoresis systems were used and in all the cases 0.5 cm × 5 cm tubes were used.

System 1: disc electrophoresis was carried out on 7.5% polyacrylamide gels in 30 mM-Tris/5,5'-diethylbarbituric acid as electrophoretic buffer, pH 7.0, for 1 h at 3.5 mA/tube (Maurer, 1971). The gels were stained for acid phosphatase activity by the method of Barka (1961).

System 2: electrophoresis was performed on 7.5% polyacrylamide gels in 5 mM-Tris/glycine as electrophoretic buffer, pH 8.3, for 3 h at 3.5 mA/gel (Maurer, 1971). The proteins were stained by Coomassie Brilliant Blue (0.1% in 10%, v/v, acetic acid) for 2 h at 70°C. The gels were destained in methanol/acetic acid/water (4:1:15, by vol.) for 16 h at 37°C.

System 3: this system is identical with system 1 except that the gels and electrophoretic buffer contained 0.1% SDS. The top cathodic buffer is constituted by 30 mM-Tris/5,5'-diethylbarbituric acid, pH 7.5, and the anodic buffer consisted of 0.1 M-Tris/HCl, pH 6.8. The following standards were used for molecular-weight determination: horse myoglobin (17800), chymotrypsinogen A (25000), ovalbumin (45000) and bovine serum albumin (67000).

Ultracentrifugation on continuous glycerol gradient

The molecular weight of the purified kidney-cortex acid phosphatase was estimated by a glycerol-density-gradient sedimentation method. The known molecular-weight markers (aldolase, 147000, cata-

lase, 240000, and haemoglobin, 64000) and the purified acidic and basic forms of tartrate-sensitive acid phosphatase were layered on a 12–40% glycerol gradient and centrifuged in a Spinco model L 5-65 ultracentrifuge at 38000 rev./min for 17h with rotor SW-41. After centrifugation, 0.4ml fractions were collected in a gradient collector (Auto Densi Flow IIC, Searle) by puncturing the top of the centrifuge tube. The enzyme was revealed by its activity and the standard proteins by the method of Lowry *et al.* (1951).

Effect of neuraminidase on the acidic form of rabbit kidney-cortex tartrate-sensitive acid phosphatase

Portions of the purified acid phosphatase-A containing about 0.2 unit of activity (0.28 mg) were incubated with neuraminidase from *Clostridium perfringens* (0.2 mg/ml) or *Vibrio cholerae* (0.2 ml/ml) for 3 and 6h at 37°C in 50 mM-citrate/phosphate buffer, pH 5.6, containing 20 mM-CaCl₂. For controls, tubes containing all ingredients except neuraminidase were similarly incubated or stored at 4°C. Protein was measured by the method of Lowry *et al.* (1951) and free neuraminic acid by the method of Warren (1959). Portions (50 µl) of the fractions so treated were electrophoresed at 4°C in system 1. Neuraminidase without added acid phosphatase-A was electrophoresed as an additional control.

Results

Tartrate-sensitive acid phosphatases preparation

A summary of a typical preparative scheme for acidic and basic forms of tartrate-sensitive acid phosphatase from rabbit kidney cortex is presented in Table 1. Sephadex G-150 chromatography of final

extract resulted in three peaks of acid phosphatase activity. Peak I represents 20% of acid phosphatase activity found in the final extract. It is worth mentioning here that when gel filtration was carried out in the presence of dithiothreitol the specific activity of peak I did not change, whereas the specific activity of peak II increased considerably.

Peak I, when subjected to polyacrylamide-gel electrophoresis in system 1, showed two bands of acid phosphatase activity: a fast-moving band (acidic form, acid phosphatase-A) and a slow-moving band (basic form, acid phosphatase-B). Of the protein of peak I, 94% was completely adsorbed on the SP-Sephadex column. The acid phosphatase activity was eluted with 0.15 M-NaCl as a sharp peak having only 9% of the protein applied to the column. Polyacrylamide-gel electrophoresis, in system 1, again showed that the enzyme from the SP-Sephadex column had the acidic and basic forms. The second SP-Sephadex chromatography with pH-gradient elution allowed the partial separation of acidic and basic forms of tartrate-sensitive acid phosphatase. The complete separation of acidic and basic forms was achieved on the DEAE-cellulose column. Obviously acid phosphatase-B was not adsorbed, whereas the acid phosphatase-A was completely retained. Washing with 0.1 M-NaCl resulted in complete elution of acid phosphatase-A from the DEAE-cellulose column. Concanavalin A-Sepharose column chromatography constituted the last purification step for the acidic and basic forms of tartrate-sensitive acid phosphatase with specific activities of 12.0 and 0.7 µmol/min per mg of protein respectively (Table 1).

The basic form represents the major part of tartrate-sensitive acid phosphatase (87–93%), as judged after the different purification steps, which allowed the separation of acid phosphatases -A and -B

Table 1. Summary of a typical preparative scheme for acidic and basic forms of tartrate-sensitive acid phosphatase from rabbit kidney cortex

One unit of enzyme activity is defined as the amount of enzyme liberating 1 µmol of *p*-nitrophenyl phosphate/min under the conditions described in the text.

Enzyme	Purification stage	Total activity (units)	Total protein (mg)	Specific activity (munits/mg)
Total acid phosphatase	Homogenate	569	20000	29
	Final extract	123.6	3433	36
Total tartrate-sensitive acid phosphatase	Sephadex G-150	23.14	538	43
	SP-Sephadex C-50	14.54	29.2	498
Acid phosphatase (basic form)	SP-Sephadex C-50	10.08	7.3	1379
	DEAE-cellulose DE-52	7.88	2.3	3426
	Concanavalin A-Sepharose	7.00	0.58	12070
Acid phosphatase (acidic form)	SP-Sephadex C-50	1.30	8.6	151
	DEAE-cellulose DE-52	0.65	1.3	500
	Concanavalin A-Sepharose	0.45	0.64	703

Fig. 6. Polyacrylamide-gel electrophoresis of purified preparation of the basic form of tartrate-sensitive acid phosphatase from rabbit kidney cortex

Enzyme (5 μ g) was electrophoresed in system 3 for gel (a) and in system 2 for gel (b). Gels were stained for proteins. Experimental conditions are described in the Materials and Methods section.

Table 2. Some kinetic parameters of acidic and basic forms of tartrate-sensitive acid phosphatase from rabbit kidney cortex with *p*-nitrophenyl phosphate as a substrate
pH optima were determined in 50mM-sodium citrate buffer, pH 3.2–6.8. Other experimental conditions are described in the text.

Parameter	Properties of acid phosphatase	
	Acidic form	Basic form
pH optimum	3.4–5.4	3.8–4.2
K_m (mM)	0.25	1.75
V_{max} (units/mg)	0.70	12.07

(Table 1). The purity of the final preparation of acid phosphatase-B was checked by polyacrylamide-gel electrophoresis in systems 2 and 3. The enzyme migrated as a single band when the gels were stained for protein (Fig. 6). The acidic form of the tartrate-sensitive acid phosphatase (7–13%) showed three additional minor bands of protein on polyacrylamide-gel electrophoresis in system 2. Scanning densitometric study of the gels indicated that the contaminating material did not exceed 5% of the total protein found in the acid phosphatase-A preparation.

Properties of acidic and basic forms of tartrate-sensitive acid phosphatase

The kinetic parameters of acid phosphatases-A and -B are summarized in Table 2. Both the forms show a broad pH optimum between 3.4 and 5.4. The

Table 3. Effect of some compounds on the acidic and basic forms of the tartrate-sensitive acid phosphatase from rabbit kidney cortex with *p*-nitrophenyl phosphate as a substrate and at pH 4.8

The assays were carried out with 10min of pre-incubation in the presence of the effector and 1.5 μ g of acid phosphatase-A or 0.3 μ g of acid phosphatase-B. Other experimental conditions are described in the Materials and Methods section.

Effector	Concn.	Relative activities	
		Acidic form	Basic form
None		100	100
L(+)-Tartrate	0.5mM	55.7	32.1
	2.5mM	27.5	9.5
	5.0mM	14.8	4.9
Sodium fluoride	2mM	100	96.5
	10mM	71.5	41.1
	25mM	31.1	17.7
NaH ₂ PO ₄	4mM	86.2	67.3
	20mM	63.8	58.3
	50mM	46.1	43.7
Urea	1M	72.7	84.5
	2M	66.0	76.4
	4M	11.0	12.7
HgCl ₂	0.5 μ M	80.3	91.2
	2.5 μ M	78.3	77.9
	5 μ M	74.7	74.4
Formaldehyde	0.5%	96.8	89.3
	2%	76.7	52.5
	5%	26.1	29.1
EDTA	2mM	102	108.3
	10mM	97.1	103
	20mM	92.3	98.2

time-activity curves of both these forms showed that the hydrolysis of *p*-nitrophenyl phosphate was directly proportional to time at least up to 3h. The apparent K_m value of acid phosphatase-B was 7 times that of the acid phosphatase-A. Further, there were considerable differences in V_{max} of acidic and basic forms of tartrate-sensitive acid phosphatase.

A study of effect of various concentrations (5–50mM) of metal ions on acid phosphatase-A and -B indicated that Ca²⁺, Cu²⁺, Zn²⁺, Mg²⁺ and Mn²⁺ had no effect on enzyme activity. Hg²⁺ at 5 μ M produces a 25% inhibition of both forms of acid phosphatase. L(+)-Tartrate and fluoride ions were more potent inhibitors of the basic form than of the acidic form (Table 3). The extents of inhibition produced by NaH₂PO₄, urea and formaldehyde were approximately the same for both forms. The effect of temperature on acidic and basic forms of tartrate-sensitive acid phosphatase is shown in Fig. 7. It is obvious that both the forms show maximum activity at 55°C. Above this temperature there were consider-

able differences in the heat-stability of acid phosphatases-A and -B. Thus at 65°C the acidic form lost 20% of its original activity whereas the basic form showed about 30% activation in its enzyme activity. At 70°C and compared with the reaction rate at 37°C the acidic form retained only 10% of its activity, whereas the basic form retained 40% of its activity.

Substrate specificity of acidic and basic forms of tartrate-sensitive acid phosphatase

The relative hydrolytic rates of different phosphate esters at 9mM are shown in Table 4. Expressed as units/mg of protein the basic form hydrolysed at a faster rate most of the physiological and non-physiological substrates than the acidic form. D-Ribose 5'-phosphate is the only one that was a better substrate for the acidic form compared with the basic form of the enzyme. Both forms were inactive with ATP as a substrate.

Relative to the *p*-nitrophenyl phosphate hydrolysis rate, at 5mM the best substrate for the acidic form was pyridoxal 5'-phosphate (75%). Then followed in decreasing order α -naphthyl phosphate, CMP, GMP, UMP, DL-glycerol 2-phosphate, AMP, D-ribose 5'-phosphate, phosphoenolpyruvate and D-fructose 1,6-bisphosphate (54.8-33.7%). On the other hand, only α -naphthyl phosphate, CMP and phosphoenolpyruvate (61.1-40.8%) were good substrates for the basic form compared with the *p*-nitrophenyl phosphate hydrolysis rate.

Molecular weight of acidic and basic form of tartrate-sensitive acid phosphatase

The molecular weight of the basic form was estimated to be about 64000±3000 by SDS/poly-

acrylamide-gel electrophoresis, as judged by the migration of standard proteins. Linearity of marker proteins was in relation to log (mol.wt.). On the other hand, ultracentrifugation on a continuous glycerol gradient indicated a mol.wt. of 101000±4000 (Fig. 8). In addition, mixed preparations of acidic

Fig. 7. Effect of temperature on acidic and basic forms of tartrate-sensitive acid phosphatase from rabbit kidney cortex

For each temperature a separate measurement was made. Incubations were carried out for 30min with 1.5µg of the acidic form (■) and 0.25µg of the basic form (●) of the enzyme by keeping the enzymes at different chosen temperatures (37-70°C). The other experimental conditions are described in the Materials and Methods section.

Table 4. Physiological and non-physiological substrate specificity of the acidic and basic forms of the tartrate-sensitive acid phosphatase from rabbit kidney cortex

The enzymes were assayed as described in the Materials and Methods section. Each substrate was at 9mM concentration.

Substrate	Specific activity (units/mg of protein)		Basic form/acidic form
	Basic form	Acidic form	
<i>p</i> -Nitrophenyl phosphate	12.10	0.70	17.3
DL-Glycerol 2-phosphate	10.20	0.33	31
α -Naphthyl phosphate	7.39	0.50	14.8
D-Fructose 1,6-bisphosphate	1.63	0.24	6.8
D-Glucose 6-phosphate	0.63	0	
2,3-Bisphosphoglyceric acid	0.07	0	
Phosphoenolpyruvate	4.94	0.24	20.6
D-Ribose 5-phosphate	0.04	0.24	0.2
Pyridoxal 5'-phosphate	1.02	0.53	1.9
AMP	2.70	0.29	9.3
GMP	1.60	0.37	4.3
UMP	2.71	0.33	0.2
CMP	5.70	0.38	15
ATP	0	0	

Fig. 8. Molecular-weight estimation of tartrate-sensitive acid phosphatase by ultracentrifugation on a continuous glycerol gradient

Three glycerol gradients were poured simultaneously with additions as follows. First gradient (■), 1 mg of haemoglobin (1: mol.wt. 64000) and 2.5 mg of aldolase (2: mol.wt. 147000) were layered; second gradient (▲), 2.5 mg of catalase (3: mol.wt. 240000) was layered; third gradient (●), 50 μ g of basic-form acid phosphatase (B-AP) was layered. Other experimental conditions are described in the Materials and Methods section. ----, Glycerol gradient. Plot of the logarithm of the molecular weight as a function of the number of the fraction containing maximum protein or activity is shown in the inset to the Figure.

and basic forms gave a single peak of activity with this method.

Treatment of the acidic form of tartrate-sensitive acid phosphatase with neuraminidases

The treatment of the acidic form of tartrate-sensitive acid phosphatase with bacterial neuraminidases does not affect the activity or mobility of this form on polyacrylamide gel. Furthermore, the treatment with two types of neuraminidases did not show any liberation of free neuraminic acid. Studies made by Goldstone *et al.* (1971) have indicated that some basic forms of lysosomal hydrolases are merely the asialo derivatives of the corresponding acidic form.

Discussion

The present paper is the first report on the purification and properties of two forms of soluble tartrate-sensitive acid phosphatase from rabbit kidney cortex. It is very difficult to estimate the exact amount of soluble tartrate-sensitive acid phosphatase in kidney homogenate. But, knowing that kidney-

cortex soluble acid phosphatase represents 50% of total activity, that 50% of soluble cortical acid phosphatase activity was recovered and that tartrate-sensitive acid phosphatase represents 15–20% of the soluble activity as judged after Sephadex G-150 column chromatography, we can estimate that tartrate-sensitive acid phosphatase represents 8–10% of the total activity present in the homogenate, that is to say about 50 units for 18 rabbits (Table 1). Thus the recovery of soluble tartrate-sensitive acid phosphatase is about 10–15%. The method described for the purification of this acid phosphatase needs few comments. The retention of enzyme on concanavalin A-Sepharose and its elution by methyl α -D-mannoside indicate that the binding of acid phosphatase to concanavalin A-Sepharose is through the carbohydrate moiety. It must be stressed here that the elution of acid phosphatase from the concanavalin A-Sepharose column was dependent on temperature. The maximum elution was obtained with 0.6M- α -methyl mannoside whatever the temperature used. With this concentration the enzyme activity recovered is 4–5 times higher at 20–25°C than at 4°C. Van Etten & Saini (1977) prepared homogeneous human prostate acid phosphatase by using concanavalin A-Sepharose chromatography. These authors did not mention the effect of temperature on the elution of prostatic tartrate-sensitive acid phosphatase from a concanavalin A-Sepharose column. But this phenomenon has been recently reported for *N*-acetylhexosaminidase (Brattain *et al.*, 1977).

The molecular weight of both forms of tartrate-sensitive acid phosphatase was estimated to be $101\,000 \pm 4\,000$ by continuous glycerol gradient centrifugation. On SDS/polyacrylamide-gel electrophoresis, the molecular weight of the basic form was estimated to be $64\,000 \pm 3\,000$. This observation suggests that tartrate-sensitive acid phosphatases of rabbit kidney cortex are probably composed of two subunits of approximately the same size. This result concords with those obtained for tartrate-sensitive acid phosphatase from other sources. Studies on human prostatic tartrate-sensitive acid phosphatase (Luchter-Wasyl & Ostrowski, 1974) have indicated that this enzyme is a dimer with a molecular weight, as determined by sedimentation-equilibrium analysis, of $102\,000 \pm 1\,000$. A subunit size of $50\,000 \pm 2\,000$ was obtained by SDS/polyacrylamide-gel electrophoresis. Lam *et al.* (1973) also reported that human prostate acid phosphatase had a mol.wt. of 100000. Human liver tartrate-sensitive acid phosphatase has a mol.wt. of 107000 (Rehkop & Van Etten, 1975). Human placenta tartrate-sensitive acid phosphatase has a mol.wt. of 105000 (DiPietro & Zengerle, 1967).

Relative to the *p*-nitrophenyl phosphate hydrolysis rate, the acidic form has a much larger activity

spectrum towards several physiological and non-physiological phosphate esters than the basic form. This latter form shows appreciable activity only towards glycerol 2-phosphate, α -naphthyl phosphate, phosphoenolpyruvate and CMP. In spite of that, the basic form remains a more potent phosphohydrolytic enzyme than the acidic form if their specific activities are considered. The basic form differs also from the acidic form in its heat-stability, K_m values towards *p*-nitrophenyl phosphate and inhibition rates by sodium tartrate and fluoride ions. The question arises whether the two forms of the enzyme are different proteins or whether one is a modified form of the other.

Studies on the heterogeneity of some acid phosphatases from different sources (Smith & Whitby, 1968; Ostrowski *et al.*, 1970; Campbell *et al.*, 1973) have indicated that multiple molecular forms may differ only in their content of various amounts of bound sialic acid. In the present study it was observed that neuraminidase treatment of tartrate-sensitive acid phosphatase from rabbit kidney cortex does not result in the conversion of the acidic form into the basic form of this enzyme. Studies from other laboratories have also demonstrated that treatment of highly purified arylsulphatase A (Graham & Roy, 1973), hexosaminidase A (Srivastava *et al.*, 1974; Tallman *et al.*, 1974) and α -galactosidase A (Beutler & Kuhl, 1972) with bacterial neuraminidase does not produce the basic forms of these enzymes. The total rat kidney lysosomal acid phosphatases show five bands on polyacrylamide-gel electrophoresis (Goldstone *et al.*, 1971). Only three bands are strongly affected by the bacterial neuraminidase treatment. The possibility is not excluded that the other bands could be tartrate-sensitive. All this together suggests that it is unlikely that some of the basic forms of lysosomal hydrolases are merely asialo derivatives of the corresponding acidic forms (Patel & Koenig, 1976).

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