A Tartrate-resistant Acid Phosphatase from Gaucher Spleen

PURIFICATION AND PROPERTIES

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The acid phosphatase activity that is elevated 3- to 6-fold in the spleen in Gaucher’s disease can be resolved into two principal isoenzymes by chromatography on sulphotropropyl-Sephadex: one tartrate-sensitive component does not bind to the resin whereas the second acid phosphatase, which is tartrate-resistant, binds to the column and can be eluted by a buffer containing 0.4 M sodium chloride.

We have isolated and characterized the L-(+)-tartrate-resistant isoenzyme of acid phosphatase (designated SP) from the spleen of a patient with adult (type 1) Gaucher’s disease. The enzyme represents approximately 50% of the total acid phosphatase activity in a homogenate of spleen prepared in the presence of Triton X-100 and has been purified 24,000-fold by chromatography on columns containing carboxymethyl-Sepharose, hydroxyapatite, Sephadex G-150, and concanavalin A-Sepharose. The specific activity of the final preparation (1490 pmol/min/mg) is one of the highest reported for an acid phosphatase from a human source. The SPn acid phosphatase isoenzyme catalyzes the hydrolysis of the artificial substrates 4-methylumbelliferyl phosphate and p-nitrophenyl phosphate, as well as nucleoside tri- and diphosphates. The Gaucher spleen acid phosphatase also catalyzes the dephosphorylation of phosvitin and erythrocyte membrane phosphoproteins including spectrin. The enzyme is inhibited by molybdate (K, 0.002 mm), cupric ion, fluoride (K, 1.0 mm), diithionite (K, 0.5 mm), and ferrous ion (K, 1.5 mm). The enzyme has the following properties: 1) pH optimum, 5.5; 2) K, 0.90 mm on 4-methylumbelliferyl phosphate; 3) pl, 8.5; 4) Smax, 3.3; 5) M, 33,000.

Gaucher’s disease is a lysosomal storage disease in which glucocerebroside accumulates in the form of highly characteristic deposits in the lysosomes of reticuloendothelial cells (1-5) because of a deficiency of the enzyme glucocerebrosidase (6, 7). In the adult non-neuronopathic form (type 1) of Gaucher’s disease, hepatosplenomegaly is the major visceral manifestation and anemia, thrombocytopenia, and erosion of the cortices of the long bones are common complications (1, 5). In infantile (type 2) and juvenile (type 3) Gaucher’s disease, there are not only visceral complications but also extensive central nervous system involvement resulting in profound neurological disturbances. Although patients in all three categories exhibit profoundly decreased levels of tissue glucocerebrosidase, the biochemical basis that distinguishes the various clinical presentations of the disease has not yet been identified.

A nearly constant finding in all patients with Gaucher’s disease is the occurrence of elevated serum acid phosphatase activity (8). Increased serum acid phosphatase activity in Gaucher’s disease was first reported in 1956 by Tuchman et al. (9) when they noted elevated acid phosphatase activity in the serum of a 52-year-old woman with a lytic bone lesion in whom the diagnosis of Gaucher’s disease was confirmed by positive pathological findings in a bone marrow aspirate drawn 18 years after splenectomy. Using homogenates of spleen as a source of enzyme, Crocker and Landing (8) confirmed that tissue acid phosphatase is also elevated in Gaucher’s disease. In the more than 20 years that have transpired since Tuchman and coworkers reported their observations, elevated serum acid phosphatase values have been documented in several hundred cases of Gaucher’s disease. In fact, the association of increased serum acid phosphatase activity with all three clinical forms of Gaucher’s disease is such a common finding that a number of clinical laboratories now employ some type of serum acid phosphatase determination as a noninvasive screening procedure when considering the diagnosis of Gaucher’s disease in infants, children, or adults (10). Despite the diagnostic value and potential pathophysiological significance of the acid phosphatase activity that is elevated in serum and stored organs of patients with Gaucher’s disease, neither the serum nor tissue enzymes have been isolated and characterized.

The spleen and serum of patients with Gaucher’s disease in fact contain elevations in two prominent forms of acid phosphatase that can be readily resolved by chromatography on columns of SPn-Sephadex (11). One acid phosphatase isoenzyme (designated SP) that is present in detergent (Triton X-100) extracts of Gaucher spleen, does not bind to the cation-exchange resin and has the following properties: 1) pH optimum, 4.5; 2) inhibited by L-(+)-tartrate and high concentrations of β-mercaptoethanol, 3) resistant to inhibition by sodium diithionite. The acid phosphatase isoenzyme that binds to the column (designated SPn) has properties similar to those of the principal acid phosphatase isoenzyme present in serum from patients with Gaucher’s disease: 1) pH optimum, 5.5; 2) inhibited by sodium dithionite, 3) resistant to inhibition by L-(+)-tartrate. The present report describes the purification and properties of the tartrate-resistant SPn phosphatase from the spleen of a patient with Gaucher’s disease.1

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1 Enzyme Nomenclature (EC 3.1.3.2) orthophosphoric-monoester phosphohydrolase, acid optimum.
2 The abbreviations used are: SP, sulphotropropyl; MUP, 4-methylumbelliferyl phosphate; SDS, sodium dodecyl sulfate.
3 Portions of this paper (including "Experimental Procedures").
Spleen Acid Phosphatase in Gaucher's Disease

Table IV

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Figure</th>
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<tbody>
<tr>
<td>pH optimum</td>
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</tr>
<tr>
<td>$K_v$ (MUP, mM)</td>
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</tr>
<tr>
<td>Isoelectric point (pI)</td>
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<td>11</td>
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<td>s_{av}</td>
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<td>Subunit molecular weights</td>
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<td></td>
<td>16,300</td>
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<td>Frictional coefficient (f/f₀)</td>
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The spleen in Gaucher's disease contains approximately equal amounts of two distinct forms of acid phosphatase. The two prominent isoenzymes are initially resolved from a detergent (e.g., Triton X-100) extract of Gaucher spleen by chromatography on SP-Sephadex. The isoenzyme designated SP1 does not bind to the cation-exchange resin while the very basic SPII acid phosphatase binds tenaciously to this resin and is eluted only with buffer containing 0.4 M NaCl. In the present report, we describe the purification and properties of the SP1 acid phosphatase.

The final preparation of the SP1 acid phosphatase contained two species of the enzyme that could only be resolved by prolonged electrophoresis in 15% polyacrylamide disc gels (Fig. 4). The ability of the enzyme precluded an attempt at the separation and further purification of the two species by preparative isoelectric focusing techniques. Similar microheterogeneity has been observed in highly purified preparations of prostatic acid phosphatase (39, 40). However, unlike that of the prostatic enzyme, the microheterogeneity in the SP1 acid phosphatase is apparently not the result of a disparity in the sialic acid content of the two electrophoretic species.

The SP1 acid phosphatase is a relatively small ($M_r = 33,000$), highly basic (pI = 8.5) protein with one of the highest specific activities (1480 units/mg) of an acid phosphatase from any human source reported to date. A summary of the physical and kinetic properties of the enzyme is provided in Table IV.

The enzyme appears to be a glycoprotein on the basis of its affinity for Con A-Sepharose and because its electrophoretic mobility is altered by treatment with neuraminidase. Preliminary results of SDS-polyacrylamide gel electrophoresis indicate that the enzyme is composed of nonidentical subunits with molecular weights of 16,000 and 20,000. However, the presence of 6.25 M urea and 5% (v/v) 8-mercaptoethanol does not affect the apparent molecular weight or activity of the enzyme.

The Gaucher spleen enzyme has a pH optimum of 5.5, is resistant to inhibition by L-(-)-tartrate, and has the cathodal electrophoretic mobility of a type 5 acid phosphatase according to the isoenzyme nomenclature of Li et al. (23). Type 5 acid phosphatase is undetectable in normal human spleen and most other tissues but is the predominant isoenzyme in the spleen of patients with leukemic reticuloendotheliosis or hairy cell leukemia (23, 41, 42). Purified 64,000-dalton, L-(-)-tartrate-resistant, type 5 acid phosphatase from the spleen of hairy cell leukemia has a specific activity of 133 units/mg when assayed using p-nitrophenyl phosphate as the substrate (43). The acid phosphatase of the hairy cell exhibits the same pH optimum and essentially the same substrate specificity as the SP1 acid phosphatase of the Gaucher cell with the notable exception that inorganic pyrophosphate is an effective substrate only for the enzyme from hairy cells. It is interesting that the native SP1 isoenzyme is approximately half the size of the acid phosphatase from hairy cells. It would be worthwhile to compare the relationship between the type 5 isoenzyme of the Gaucher cell and the hairy cell using immunological methods.

Some of the properties of the SP1 acid phosphatase isoenzyme are shared by the 32,000-dalton acid phosphatase of pig uterine fluid (44) and the 23,000-dalton bovine spleen phosphoprotein phosphatase (45-47), particularly with regard to the ability to catalyze the hydrolysis of phosphate from phosphoproteins. Both the pig uterine and bovine spleen enzymes are highly basic proteins and each contains 1 atom of iron/molecule of enzyme (44-48). Due to their iron content, these enzymes are purple in color and exhibit a strong absorption maximum at 550 nm. The final preparation of acid phosphatase from Gaucher spleen did not have a blue-purple color but since only microgram quantities of the protein were available to us, direct assessment of the iron content of the enzyme was not possible.

The specific activity of the purified uterine acid phosphatase was demonstrated to be 40 units/mg at pH 5.0 with the substrate p-nitrophenyl phosphate and only 0.6 unit/mg with the phosphoprotein posvin (44). Various preparations of the bovine spleen phosphatase have been reported as having specific activities ranging from 6 to 50 units/mg at pH 6.0 when measured with the phosphoprotein substrate casein (45, 47) and 38 units/mg when determined using ATP as substrate (47). The specific activity exhibited by the SP1 acid phosphatase from Gaucher spleen on phosvitin is 60 units/mg, a value comparable to those reported for the bovine spleen protein phosphatase, but its activity on substrates such as p-nitrophenyl phosphate and nucleoside triphosphates is considerably higher (Table II).

Bovine spleen acid phosphatase exhibits a substrate specificity similar to that of the SP1 acid phosphatase: both enzymes prefer artificial aryl phosphate compounds (e.g., p-nitrophenyl phosphate or 8-naphthylphosphate), nucleoside tri- and diphosphates, phosphoenolpyruvate, and phosphoproteins, while the phosphoric esters of aliphatic-tocohols such as glycerol phosphate, nucleoside monophosphates, sugar phosphates, and phosphoserine are poor substrates. However, bovine spleen protein phosphatase catalyzes the hydrolysis of sodium pyrophosphate (47); whereas inorganic pyrophosphate is a very poor substrate for the SP1 acid phosphatase from Gaucher spleen.

An interesting anomaly concerning the substrate specificity of the bovine and Gaucher spleen phosphatases is that the efficient cleavage of phosphoserine residues present in the phosphoprotein phosphatase is not observed with the alky monooester, O-phospho-L-serine. The 110 phosphoserine residues in phosvitin are grouped in long runs of six to eight amino acids that terminate in a basic amino acid, generally lysine (30). The secondary structure generated by these phosphate-rich centers may represent an important determinant in the recognition of phosphoprotein substrates by protein phosphatases. The participation of the terminal lysine residue as a transient phosphoryl intermediate during the hydrolysis of serine phosphates has also been hypothesized (30). In terms of substrate concentration, the dephosphorylation of phosvitin by the SP1 acid phosphatase from Gaucher spleen did not exhibit linear Michaelis-Menten kinetics but, rather, was sig-
moidal in nature. The enzymatic removal of phosphate residues from the phosphoprotein therefore appears to be a sequential or cooperative process. Similar sigmoidal kinetics were observed for the dephosphorylation of β-casein by a phosphoprotein phosphatase from mouse liver (49).

The effects of various compounds on the SP_H isoform of acid phosphatase in Gaucher spleen and its contribution to the pathology of Gaucher’s disease is noteworthy in this regard that the dephosphorylation of phosphoprotein phosphatase from mouse liver (49).

The results of some recent studies by Kaplan et al. (54) have shown that the phosphorylated mannosyl residues of glycoprotein lysosomal hydrolases are important determinants for the recognition and uptake of the enzymes. The cleavage of the phosphorylated mannosyl residues by intracellular phosphatases suggests another possible role for the SP_H isoenzyme.

Although the SP_H acid phosphatase did not catalyze the hydrolysis of phosphate from either mannose 6-phosphate or the phosphorylated mannosyl polymer, yeast mann (0.3% phosphatase w/w), it is possible that other structural determinants of lysosomal enzymes may render the mannosyl-phosphate residues labile to cleavage by the acid phosphatase of the Gaucher cell. In this regard, it would be useful to investigate the ability of the SP_H acid phosphatase to hydrolyze phosphate from phosphorylated lysosomal enzymes.

Although the intracellular location of the SP_H acid phosphatase within the Gaucher cell has not been determined, the results of several studies suggest that the enzyme may exist outside of the lysosome. Hibbs et al. (55) identified acid phosphatase activity in the intertubular matrix of the cytoplasmic storage bodies in elements of the endoplasmic reticulum that were contiguous with the deposits of the developing Gaucher cell. More recently, Chambers et al. (11) found that purified storage deposits of the Gaucher cell contained exclusively the L(+) tartrate-labile SP_H acid phosphatase.

The organ that is the principal source of the elevated serum acid phosphatase in patients with Gaucher’s disease has not been defined. However, the major acid phosphatase present in the serum of patients with this lipid storage disorder has several properties that are also shared by the SP_H isoform of acid phosphatase. It is possible that the SP_H acid phosphatase is confined to the endoplasmic reticulum. As fresh tissue becomes available, the subcellular location of each of the isoenzymes of acid phosphatase will be determined.

The function of the Gaucher spleen phosphatase and its contribution to the pathology of Gaucher’s disease remains unclear. However, the substrate specificity of the enzyme provides certain clues. For example, the considerable capacity of Gaucher cell acid phosphatase to hydrolyze nucleoside di- and triphosphates could severely disrupt the metabolism and energy charge of the spleen cell if the nucleotide pools were accessible to the enzyme.

In order to explore a possible physiological function of the SP_H acid phosphatase as a phosphoprotein phosphatase in Gaucher spleen and its contribution to the pathology of Gaucher’s disease, we have investigated the release of phosphate groups from erythrocyte membrane phosphoproteins. Both normal and Gaucher spleen cells are involved in the phagocytosis and degradation of erythrocytes. The Gaucher cell is often described as containing erythropagosomes (1, 2, 52) and erythrocyte membrane lipids contribute significant quantities of precursors to the glucocerebroside that accumulates in the storage cell (1, 2). The ability of the SP_H acid phosphatase to hydrolyze phosphate from erythrocyte membrane proteins (Fig. 14) suggests a possible role for the enzyme in the degradation of the phagocytized erythrocyte. The dephosphorylation of phosphoproteins by the action of a phosphatase may increase the susceptibility of these proteins to subsequent attack by lysosomal proteases. It is noteworthy in this regard that the dephosphorylation of phosvitin renders the protein more sensitive to proteolysis by trypsin (53). The SP_H acid phosphatase may also function to modulate the activity of specific enzymes through dephosphorylation.

The results of recent studies by Kaplan et al. (54) have shown that the phosphorylated mannosyl residues of glycoprotein lysosomal hydrolases are important determinants for the recognition and uptake of the enzymes. The cleavage of the phosphorylated mannosyl residues by intracellular phosphatases suggests another possible role for the SP_H isoenzyme.
REFERENCES

25. Porath, J. (1963) Pure Appl. Chem. 6, 233-244
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Table 1. Summary of the polarization of the H₂O-merophosphate

<table>
<thead>
<tr>
<th>Sample</th>
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<th>Activity/Weight</th>
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<tr>
<td>1</td>
<td>1.2</td>
<td>0.003</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
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<td>0.002</td>
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</tr>
<tr>
<td>3</td>
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<tr>
<td>5</td>
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*All values are calculated on the basis of 125 mm of the H₂O-merophosphate and 12 meq of the inorganic phosphate. The results are presented in µg and are represented as the mean of six determinations ± S.E. of the mean.

**Note:** The table above shows the summary of the polarization of the H₂O-merophosphate for different samples. The activity is measured in µg, and the activity/weight is presented as a ratio. The absorbance values are also included for each sample.

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**Graphology:**

1. **Graph 1:** Chromatography of the H₂O acid phosphatase in Gaucher’s disease. The graph illustrates the separation of the H₂O acid phosphatase component in the disease. The separation is based on the molecular weight of the enzyme, with the elution time increasing from left to right.

2. **Graph 2:** Polarization of the H₂O acid phosphatase. The graph shows the polarization of the enzyme, with the absorbance values increasing as the polarization increases.

3. **Graph 3:** The ratio of the polarization of the H₂O acid phosphatase to the molecular weight of the enzyme. The graph demonstrates the relationship between the polarization and the molecular weight, indicating a linear correlation.

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**Discussion:**

The results presented in this paper indicate that the H₂O acid phosphatase in Gaucher’s disease exhibits a higher polarization compared to the control samples. This finding supports the hypothesis that the polarization of the enzyme is altered in Gaucher’s disease, which could have implications for the disease’s diagnosis and management.

**Conclusion:**

Further studies are needed to validate these findings and to elucidate the underlying mechanisms that drive the polarization changes in Gaucher’s disease. This could lead to the development of novel therapeutic strategies for this disease.


Spleen Acid Phosphatase in Gaucher's Disease

![Graph 1](image1.png)  
**Figure 1:** The effect of temperature on the activity of spleen acid phosphatase. The enzyme activity is measured in the absence of substrate. The enzyme is heat-stable and retains its activity at 60°C. The enzyme is not affected by the presence of 1 mM EDTA, a known inhibitor of metalloproteases.

![Graph 2](image2.png)  
**Figure 2:** Determination of the sedimentation coefficient of the spleen acid phosphatase. The enzyme was isolated from the spleen of a patient with Gaucher's disease and subjected to velocity sedimentation in a sucrose gradient. The sedimentation coefficient was determined to be 3.5 Svedberg units (S). The enzyme is a monomer and exhibits a similar sedimentation behavior in the presence or absence of substrate.

![Graph 3](image3.png)  
**Figure 3:** The sedimentation coefficient of the spleen acid phosphatase was determined at various temperatures. The enzyme is stable over a wide range of temperatures and retains its activity at 4°C and 37°C.

![Graph 4](image4.png)  
**Figure 4:** Determination of the molecular weight of the spleen acid phosphatase. The enzyme was electrophoresed on a sodium dodecyl sulfate-polyacrylamide gel and stained with Coomassie Blue. The molecular weight was determined to be 55,000 daltons (Da). The enzyme is a single polypeptide chain with a mol wt of approximately 55,000 Da.

![Graph 5](image5.png)  
**Figure 5:** Determination of the molecular weight of the spleen acid phosphatase. The enzyme was electrophoresed on a sodium dodecyl sulfate-polyacrylamide gel and stained with Coomassie Blue. The molecular weight was determined to be 55,000 daltons (Da). The enzyme is a single polypeptide chain with a mol wt of approximately 55,000 Da.

![Graph 6](image6.png)  
**Figure 6:** Determination of the molecular weight of the spleen acid phosphatase. The enzyme was electrophoresed on a sodium dodecyl sulfate-polyacrylamide gel and stained with Coomassie Blue. The molecular weight was determined to be 55,000 daltons (Da). The enzyme is a single polypeptide chain with a mol wt of approximately 55,000 Da.

![Graph 7](image7.png)  
**Figure 7:** Determination of the molecular weight of the spleen acid phosphatase. The enzyme was electrophoresed on a sodium dodecyl sulfate-polyacrylamide gel and stained with Coomassie Blue. The molecular weight was determined to be 55,000 daltons (Da). The enzyme is a single polypeptide chain with a mol wt of approximately 55,000 Da.
Spleen Acid Phosphatase in Gaucher’s Disease

In an effort to identify a pathological phenomenon associated with the Gaucher’s disease, we performed the following experiments.

Figure 1: Graph showing the relationship between enzyme activity and substrate concentration.

Table 1: Summary of experimental results.

The graph illustrates the enzymatic activity as a function of substrate concentration. The data points were fitted to a Michaelis-Menten kinetic model, and the values were obtained from multiple trials.

Table 2: Comparison of enzyme activities under different conditions.

The results indicate a significant increase in enzyme activity under certain conditions, which could be relevant for further study.

Figure 2: Graph showing the inhibition of enzyme activity by various inhibitors.

Inhibition of enzyme activity by the addition of inhibitors is shown in the graph. The inhibition constants (K_i) were calculated using the Lineweaver-Burk plot.

Table 3: List of inhibitors and their corresponding K_i values.

The inhibitors were screened for their ability to inhibit the enzyme activity. The highest inhibition was observed with compound A, which showed a K_i value of 0.02 M.

Conclusion:

The study provides evidence for the potential role of spleen acid phosphatase in Gaucher’s disease. Further research is needed to understand the mechanisms involved and to explore therapeutic strategies.

References:

1. [Citation 1]

2. [Citation 2]

3. [Citation 3]