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A GOLGI APPARATUS ACID PHOSPHATASE

S. E. NYQUIST* AND H. H. MOLLENHAUER**
Charles F. Kettering Research Laboratory, Yellow Springs, Ohio 45387 (U.S.A.)

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SUMMARY

Isolated Golgi apparatus fractions and total homogenates from rat testis were examined for acid phosphatase activity (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2). A comparison of pH optima and substrate specificity of the acid phosphatase activity both in homogenates and in the purified Golgi apparatus fraction suggested an enrichment of certain enzymes during the fractionation procedure. Polyacrylamide disc gel electrophoretic patterns of Triton X-100 solubilized enzyme stained for acid phosphatase activity indicated that the Golgi apparatus possessed an unique acid phosphatase. Chromatography of the Triton X-100 solubilized Golgi apparatus proteins on DEAE-cellulose columns also suggested the presence of an unique Golgi apparatus acid phosphatase. Fractions off the DEAE-cellulose column containing this acid phosphatase were further examined and found to be inhibited by low levels of both NaF and sodium tartrate. This enzyme was also partially inhibited by Cu^{2+} but not Cd^{2+} or formaldehyde; exhibited a broad pH optima between pH 3.5 and 6.0 and was stable at incubation temperatures up to 40 °C.

INTRODUCTION

Acid phosphatase activity (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) was early associated with the Golgi apparatus by histochemical techniques; however other cell membranes and organelles also demonstrated considerable activity by both histochemical and cell fractionation techniques. Distribution studies of acid phosphatase enzymes were complicated by the presence of multiple enzymes as shown for a number of tissues[1–3]. Recently a study of rat testicular homogenates demonstrated the presence of at least four distinct acid phosphatase enzymes, each with characteristic properties[4]. This paper reports the results of a study of the acid phosphatase activity of isolated Golgi apparatus from rat testis germ cells. We report

* Present address: Department of Biology, Bucknell University, Lewisburg, Pa. 17837, U.S.A.
** Present address: Veterinary Toxicology and Entomology, Research Laboratory, U.S.D.A. Agricultural Research Service, P.O. Drawer GE, College Station, Texas 77840, U.S.A.

the presence and partial purification and characterization of what appears to be an acid phosphatase specific for the Golgi apparatus. A preliminary report of this work appeared earlier in abstract form[5].

METHODS

Adult male albino rats (300–450 g) were obtained from Holtzman and used in the preparation of all subcellular fractions. Golgi apparatus fractions were prepared as originally described by Cunningham *et al.*[6] and later modified[7].

Acid phosphatase activities were assayed by a number of methods. Using *p*-nitrophenyl phosphate as the substrate, the reaction mixture contained 0.1 M acetate buffer at the pH indicated, 5 mM $MgCl_2$, and 1 mM substrate in a final volume of 1 ml. The incubation was continued for 5 to 15 min and stopped by addition of 1 ml of 0.2 M NaOH. The color was read at 400 nm against a blank containing all the reaction mixture components. The absorbance of *p*-nitrophenol at 400 nm under the given conditions was determined using a *p*-nitrophenol standard.

β-Glycerophosphate (10 mM), α-naphthyl phosphate (5 mM), or β-napthyl phosphate (5 mM) were also used as substrates. These assays were carried out for 30 min and then stopped by addition of 1 ml of 10% trichloroacetic acid. Phosphates were then determined using the method of Chen *et al.*[8]. In experiments where the pH optimas were determined all pH readings were made during the course of the incubation. All values plotted are averages of triplicate determinations.

The general method of acrylamide gel electrophoresis as described by Davis[9] was used. The enzymes were layered over the gels in 0.33 M sucrose and a volume of 0.2 ml. They had previously been solubilized by suspension in a 1% Triton X-100 solution for 30 min prior to electrophoresis. 2 mA per disc was applied to effect the separation, and the temperature was maintained by packing the electrophoresis unit in ice. Acid phosphatase activity was detected by use of the coupling diazo dye Fast Garnet GBC. Following electrophoresis the gels were incubated in a mixture containing 5 mM $MgCl_2$, 5 mM α(or β)-naphthyl phosphate, 100 mM acetate buffer, pH 5.5 or 3.5, and 0.1% diazo Fast Garnet GBC dye. The incubation was continued until distinct red or black bands were observed.

For the chromatographic separation of the enzyme on DEAE-cellulose, the Golgi apparatus fraction or the total homogenate was first solubilized in 1% Triton X-100 in 0.02 M Tris–HCl buffer, pH 8.0. The insoluble material was removed by centrifugation at 100 000 $\times$ *g* for 30 min. The soluble protein was applied to a DEAE-cellulose column (1 cm $\times$ 40 cm) and washed with buffer until no Triton X-100 appeared in the eluent. Following the wash, the column was eluted with a linear NaCl gradient (0–0.5 M) in 0.02 M Tris–HCl buffer, pH 8.0, and 2-ml fractions were collected. The samples containing the acid phosphatase activity, eluting off the column at approximately 0.3 M NaCl in the Golgi apparatus fraction, were pooled and used in further studies.

For assay of this acid phosphatase enzyme a 0.25-ml aliquot of enzyme was used in a final volumn of 0.5 ml containing 0.1 M acetate buffer, pH 5.0, unless otherwise indicated. The ion inhibition studies were conducted by addition of the ions directly to the reaction mixture and the incubation was started by addition of the enzyme. The thermal denaturation study was carried out by subjecting the enzyme

to a 15-min preincubation at the temperature indicated, cooling in ice and then assaying for acid phosphatase as previously described. Proteins were determined by the method of Lowry *et al.*[10].

RESULTS

The Golgi apparatus fractions isolated from the total homogenate and used in this study (Fig. 1) consisted of relatively intact Golgi apparatus whose cisternae remained stacked and associated with numerous vesicular profiles. The purity of these fractions is comparable to that previously reported[7,11].

Studies initially made to determine the optimum conditions to assay acid

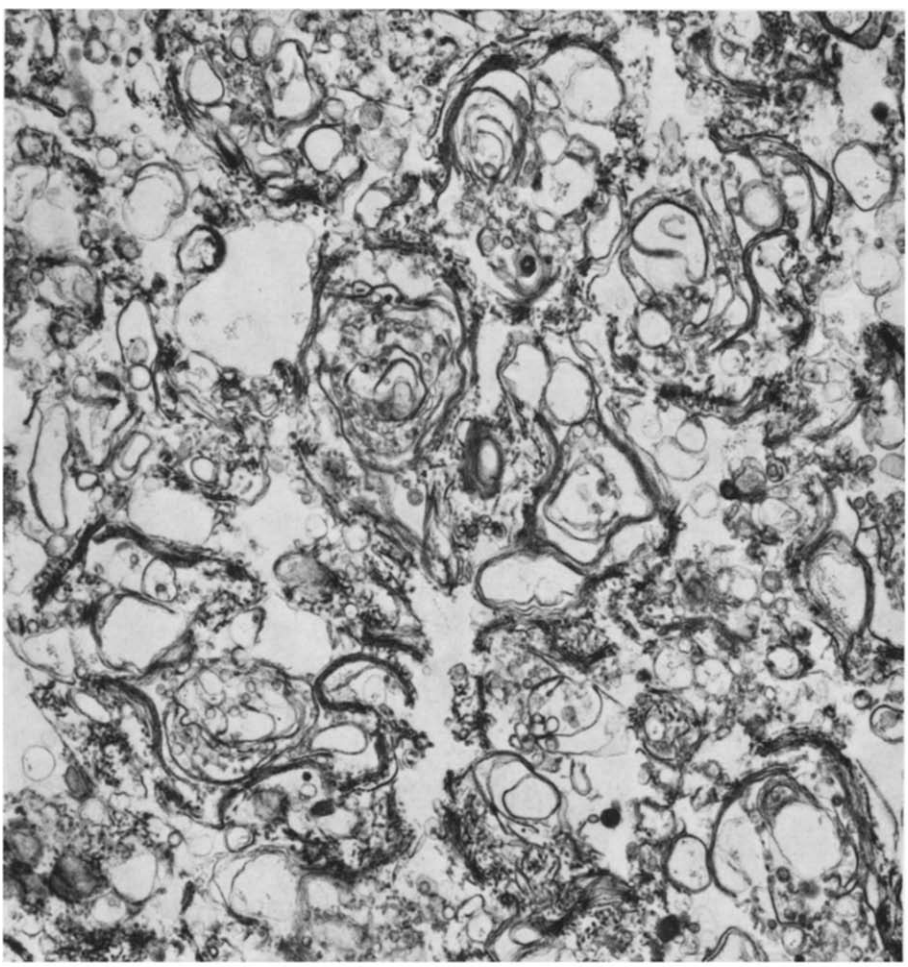

Fig. 1. Section through a pellet of isolated Golgi apparatus illustrating typical fraction purity. Note that the Golgi apparatus remain relatively intact and do not become unstacked. This is an important consideration because loss of cisternae by unstacking is accompanied by loss of the intercisternal substances and many of the associated tubules and vesicles. Such losses could result in an imbalanced evaluation of Golgi apparatus chemistry. × 13 000.

phosphatase activity indicated that optimum assay conditions depended on the tissue fraction examined and the substrate used. For example, at pH 5.5 with *p*-nitrophenyl phosphate (1 mM) as substrate, the Golgi apparatus fraction and the total homogenate showed approximately equivalent specific activities. The results of an acid phosphatase distribution study at a lower pH, 3.5 (Table I), demonstrated an increased specific activity in the Golgi apparatus fraction relative to the total homogenate. Using *α*-naphthyl phosphate as substrate a 4-fold increase in the specific activity of the Golgi apparatus over that of the homogenate was observed, with *β*-naphthyl-phosphate a 5-fold increase was observed and with *p*-nitrophenyl phosphate as substrate a 3-fold increase was observed. A 0.5% recovery of protein (Table I) and a 2–3% recovery of the total testicular acid phosphatase was observed in the Golgi apparatus fraction (Table I).

TABLE I

RECOVERY OF ACID PHOSPHATASE ACTIVITY, pH 3.5

Fraction	*Substrate*						
	α-Naphthyl phosphate		*β-Naphthyl phosphate*		*p-Nitrophenyl phosphate*		*Protein recovery*
	*Spec. act.**	*Recovery (%)*	*Spec. act.*	*Recovery (%)*	*Spec. act.*	*Recovery (%)*	*(%)*
Total homogenate	0.34	100	0.38	100	1.05	100	100
Lower pellet (2800 × *g*)	0.15	7	0.37	17	1.00	16	17
Supernatant (2800 × *g*)	0.27	41	0.41	61	1.52	82	57
Gradient supernatant (140 000 × *g*)	0.06	0	0.06	0	0.49	0	0
Gradient pellet (140 000 × *g*)	0.57	20	0.49	16	1.48	17	12
Golgi apparatus	1.32	2	2.02	3	3.36	2	0.5
% Recovery		70		0.97		117	87

* Specific activity expressed as *μ*moles/mg protein per h.

The acid phosphatase activity measured as a function of pH with various substrates (Figs 2a, 2b and 2c) showed widely varied responses when measured in the Golgi apparatus fraction or the total homogenate. Using 10 mM *β*-glycerophosphate as substrate very low activity was observed in the Golgi apparatus while the total homogenate showed a high level of activity with a maximum at pH 6.0 (Fig. 2a). Using 5 mM *α*-naphthyl phosphate as substrate the Golgi apparatus possessed greater activity than the total homogenate at all pH values observed with the difference being greatest at pH 3.5 (Fig. 2b). Using 5 mM *β*-naphthyl phosphate as substrate the homogenate showed a sharp peak of activity at pH 6.0 while the Golgi apparatus showed less activity at pH 6.0 than the total homogenate; however at pH 3.5 the Golgi apparatus possessed greater activity than the homogenate Fig. 2c).

Subsequent to the demonstration of a selective distribution of acid phosphatase enzymes during the isolation procedure, attempts were made to solubilize and sepa-

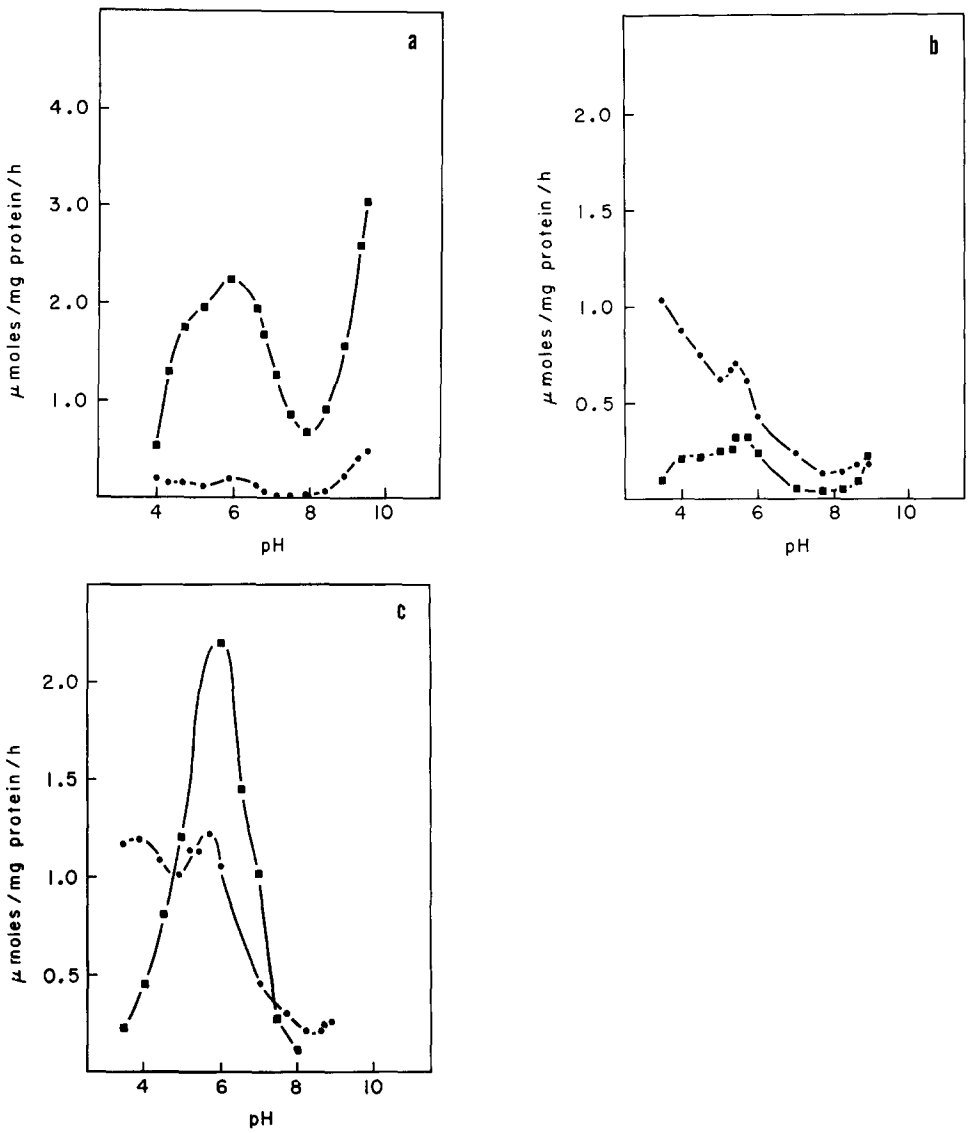

Fig. 2. Acid phosphatase activity of the total homogenate (■) and the Golgi apparatus fraction (●) as a function of pH. The substrates used were as follows: (a) 10 mM β-glycerophosphate; (b) 5 mM α-naphthylphosphate; (c) 5 mM β-naphthyl phosphate.

rate the Golgi apparatus acid phosphatase(s). Initial attempts to solubilize the acid phosphatase activity in the total homogenate by sonication resulted in only a 70% solubilization of the total acid phosphatase activity or approximately 60% of the membrane bound activity. Thus the use of detergents was investigated. Triton X-100 was chosen since the acid phosphatase activity did not appear to be inhibited by concentrations as high as 5%. By use of a 1% Triton X-100 solution, 95% of the phosphatase activity of the total homogenate was solubilized, thus its use was routinely adopted.

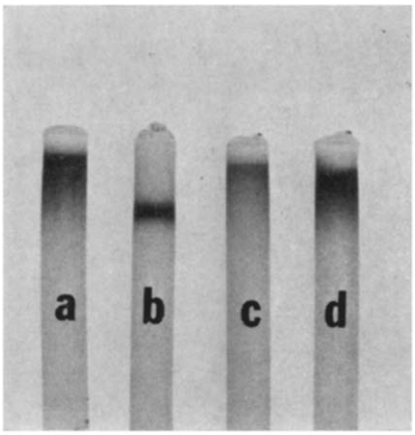

Fig. 3. Disc gel electrophoretic patterns of acid phosphatase activity. The soluble proteins applied to the gels were as follows: (a) total homogenate; (b) Golgi apparatus; (c) total soluble protein (100 000 × *g*, 1-h supernatant); (d) total particulate protein (100 000 × *g*, 1-h pellet). Acid phosphatase activity was detected using β-naphthyl phosphate as substrate at pH 5.0. Other conditions were as described in Methods.

Disc gel electrophoretic patterns of the total solubilized proteins from the Golgi apparatus and the total homogenate showed that the acid phosphatase activity of the Golgi apparatus migrated in a distinct band ahead of the more diffuse activity in the total homogenate (Fig. 3). No corresponding band was observed when an equal quantity of total homogenate protein was run parallel to the Golgi apparatus; however this was not surprising since the Golgi apparatus makes up no more than a small percentage of the total homogenate. The protein recovery of 0.5% (Table I) demonstrated a fairly high percentage recovery[6].

When Triton X-100 solubilized Golgi apparatus proteins were applied to a DEAE-cellulose column and eluted with a linear NaCl gradient, a peak of acid phosphatase activity eluted at about 03. M NaCl (Fig. 4). A phosphate containing compound of unknown identity eluted earlier in the first fractions off the column

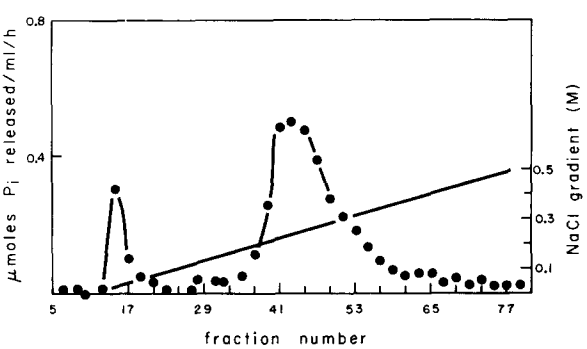

Fig. 4. Elution pattern of acid phosphatase activity off a DEAE-cellulose column. Soluble Golgi apparatus protein was applied to the column and eluted by a NaCl gradient as described in the methods. An aliquot of 0.25 ml was removed from each fraction and used directly as enzyme. Acid phosphatase activity was determined using β-naphthyl phosphate as substrate at pH 5.0. Other conditions were as described in Methods.

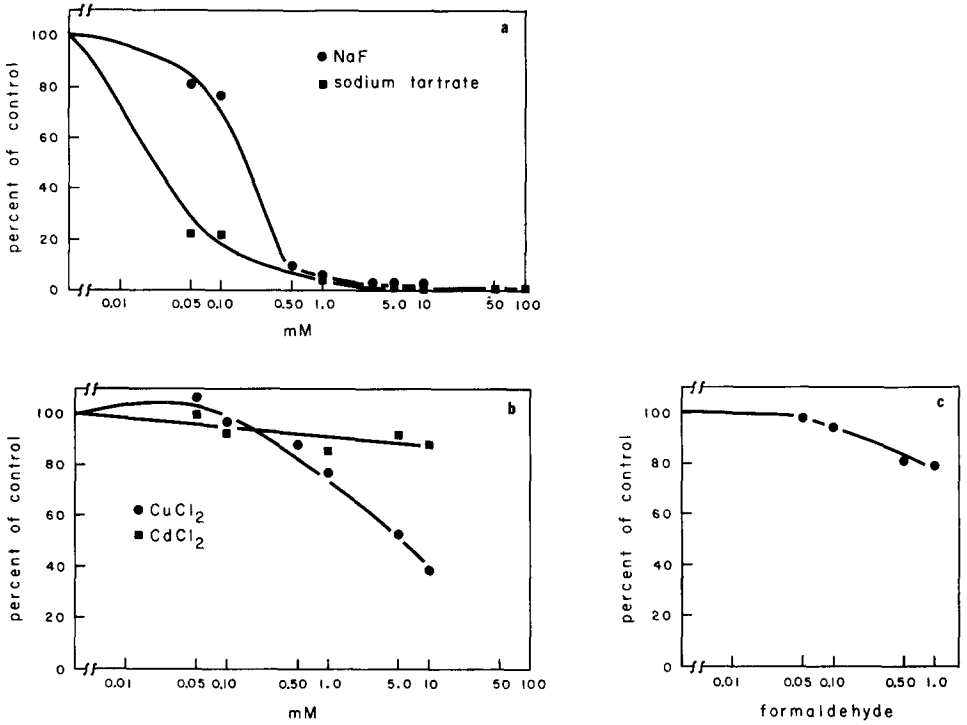

Fig. 5. Ion effects on acid phosphatase activity. The fractions off the DEAE-cellulose column which contained the Golgi apparatus acid phosphatase were pooled and used for these studies.

when either the Golgi apparatus or total homogenate proteins were used. When the total homogenate proteins were applied, very low activity was observed eluting at 0.3 M NaCl. The majority of acid phosphatase activity of the total homogenate did not bind to the column and thus came off in the buffer wash along with the Triton X-100. Some activity was also observed here in the Golgi apparatus fraction.

The fractions collected off the DEAE-cellulose column containing the Golgi

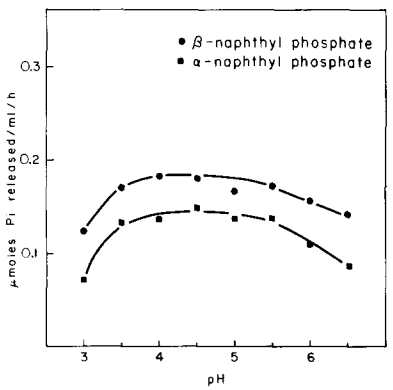

Fig. 6. Effect of pH on acid phosphatase activity. The fractions off the DEAE-cellulose column which contained the Golgi apparatus acid phosphatase were pooled and used for these studies.

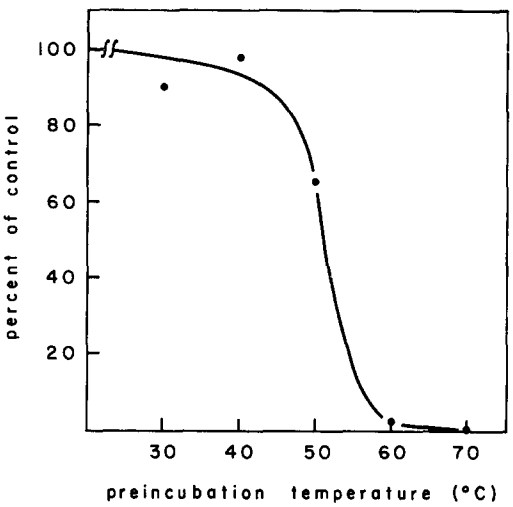

Fig. 7. Thermal denaturation curve. The fractions off the DEAE-cellulose column which contained the Golgi apparatus acid phosphatase were pooled and used for these studies. The enzyme was subjected to a 15-min preincubation at the indicated temperature, cooled in ice and then assayed for acid phosphatase activity as indicated in Methods.

apparatus acid phosphatase were pooled and used for subsequent studies of this enzyme. These fractions were stored at 4 °C (up to three days) until use with little apparent loss of activity. All glassware was thoroughly cleaned—including an acid wash—before use due to the presence of an unidentified inhibitor present on the glassware. The Golgi apparatus acid phosphatase was strongly inhibited by low levels of both NaF and sodium tartrate (Fig. 5a). Heavy metal ions showed varying effects,

TABLE II

SUBSTRATE SPECIFICITY OF A TRITON X-100 SOLUBILIZED GOLGI APPARATUS ACID PHOSPHATASE

Substrates	*Concentration (mM)*	*nmoles/ml per h*
GTP	1	40
ATP	1	14
UTP	1	12
CTP	1	39
ITP	1	0
GDP	1	6
ADP	1	4
UDP	1	6
CDP	1	1
IDP	1	61
5′-AMP	5	19
2′-AMP	5	27
CMP	5	63
β-Naphthyl phosphate	5	85
α-Naphthyl phosphate	5	60
β-Glycerophosphate	5	23
β-Glycerophosphate	25	59
Glucose 6-phosphate	10	7
p-Nitrophenyl phosphate	5	83

with 10 mM Cu^{2+} showing 60% inhibition, while the same concentration of Cd^{2+} showed little inhibition (Fig. 5b). The enzyme was resistant to formaldehyde at the concentration tested (Fig. 5c).

The enzyme showed a rather broad pH optimum using either α- or β-naphthyl phosphate as substrate (Fig. 6). The enzyme was stable when incubated for 1 h at temperatures up to 40 °C. However, a 15-min preincubation of the enzyme, at 50 °C prior to assay resulted in partial inactivation (Fig. 7). A 15-min preincubation at 60 °C resulted in total inactivation.

A number of compounds were tested as possible substrates (Table II). The enzyme appears to have a rather broad specificity, although certain nucleotides were hydrolyzed more rapidly than others.

DISCUSSION

The studies of acid phosphatase activity as a function of pH with different substrates suggested the presence of multiple acid phosphatase enzymes in intact fractions of rat testicular homogenate. The isolation of the Golgi apparatus fraction resulted in an enrichment for a particular enzyme(s). With the report by Vanha-Perttula[4] of four distinct acid phosphatase enzymes in rat testicular homogenates separated and identified by DEAE-cellulose column chromatography, an attempt was made to identify which enzymes were endogenous to the Golgi apparatus. The introduction of Triton X-100 for solubilization, however, made a direct comparison of the data somewhat difficult. The enzyme isolated off the DEAE-cellulose column did not compare in all respects with any of the four enzymes previously reported[4]. The enzyme did, however, on the basis of both the disc gel electrophoresis and the DEAE-cellulose chromatography appear to be specifically located in the Golgi apparatus.

No study to date has been made of the acid phosphatase activity washed off the column along with the Triton X-100. The acid phosphatase eluting off the DEAE-cellulose column at 0.3 M NaCl did not by itself account for the characteristics of the acid phosphatase activity observed in the intact Golgi apparatus membranes. Thus the existence of another (other than that described here) acid phosphatase enzyme(s) in the Golgi apparatus fractions seems probable.

The rat testis germ cell Golgi apparatus functions in the formation of the sperm acrosome, generally thought to be a highly specialized lysosome. The sensitivity of this Golgi apparatus acid phosphatase to inhibition by both tartrate and fluoride ions and its resistance to formaldehyde is similiar to that previously reported for many lysosomal acid phosphatases. The significance of this similarity is unknown.

The location of this enzyme within the Golgi apparatus complex has not been established. The isolated Golgi apparatus fraction consisted of a relatively intact stack of cisternae, differentiated from proximal to distal face; several classes of rough surfaced vesicles; and smooth surfaced versicles[12]. The distribution studies suggested that this Golgi apparatus acid phosphatase was not present in the acrosome as a secretory product; however the presence of an inactive form of the enzyme would not have been detected by our experiments.

Once it was determined that this enzyme appeared to be specifically located in the Golgi apparatus a study was begun of its characteristics with the hope of defining assay conditions under which it would be possible to selectively assay for this

Golgi apparatus enzyme *in vitro*. To date this has not been defined; however, the advantages of such a Golgi apparatus marker enzyme would be obvious.

The substrate specificity of this Golgi apparatus acid phosphatase was rather broad, but it did show some selectivity. No evidence, however, exists as yet about its natural substrate or its physiological significance.

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