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Daniel Rosenblum, Shirley J. Petzold

Research Article

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STUDIES OF PURIFIED ENZYMES FROM NORMAL SUBJECTS AND PATIENTS WITH POLYCYTHEMIA VERA

DANIEL ROSENBLUM and SHIRLEY J. PETZOLD

From the Department of Medicine, The Jewish Hospital of St. Louis and Washington University School of Medicine, St. Louis, Missouri 63110

ABSTRACT To characterize the biological changes which result in increased granulocyte alkaline p-nitrophenyl phosphatase activity in patients with polycythemia vera, the enzyme was purified from granule fractions of sucrose homogenates made from dextran-sedimented leukocytes of normal subjects and patients with polycythemia vera. Polycythemic blood yielded 3–10 times as much granulocyte alkaline phosphatase per 10^9 leukocytes as did normal blood. Sodium dodecyl sulfate extracts of granules were purified by DEAE-cellulose chromatography and sucrose gradient centrifugation to apparent homogeneity as judged by polyacrylamide disk gel electrophoresis. Granulocyte alkaline phosphatase from normal subjects was purified 6910-fold with a 60% yield and a specific activity of 47 U/mg. Granulocyte alkaline phosphatase from polycythemic patients was purified 1,166-fold with a 50% yield and a specific activity of 70 U/mg. The two enzymes did not differ in molecular weight; both appeared to be about 160,000 daltons by sucrose gradient centrifugation. Both appeared to be zinc metalloenzymes, in that they were specifically inhibited by o-phenanthroline. Their elution requirements when adsorbed to DEAE-cellulose suggested they were lipoproteins although the content of phosphorus was below the threshold of detection. The identity of the two enzymes was suggested by immunological studies in which antibody prepared against purified polycythemia vera enzyme gave a precipitation reaction of identity with another polycythemia vera enzyme and two pools of normal enzyme. It is possible to account for the difference in alkaline phosphatase activity between the granulocytes of patients with polycythemia vera and normal subjects by differences in the quantity of enzyme synthesized.

INTRODUCTION

Specific granules, characteristic organelles appearing in maturing granulocytes, have been identified in rabbit, guinea pig, and man by their alkaline phosphatase content (1, 2). Their physiologic role is apparently related to the phagocytic function of the granulocytes, as judged by the rapid appearance of alkaline phosphatase activity in phagosomes after fusion of specific granules with primary phagosomes in vitro (3–5). A physiological or pathological disorder has not been attributed to either absence or marked increase in specific granule alkaline phosphatase per se.

Since Wachstein's observations (6) in 1946, increased activity of granulocyte alkaline phosphatase has been found repeatedly in polycythemia vera, both in histochemically stained blood films and in extracts of washed leukocytes (7–9). The cause of this increased activity has not been elucidated. The investigations reported in this communication were intended to discover any differences between alkaline phosphatase from normal and polycythemic subjects. To the extent to which these investigations were carried out, the only difference identified is in the quantity of enzyme recovered from the granulocytes of normal and polycythemic subjects.

An essential first step, in the work presented here, was purifying alkaline phosphatase from specific granules. Preliminary experiments were performed on the alkaline phosphatase derived from guinea pig bone marrow (10). To minimize disruption of intermolecular bonds, organic solvents were avoided. In the absence of detergents, however, extracted enzyme was poorly soluble and tenaciously adherent to adsorbents. In the procedure to be described, a 6580-fold purification to a specific activity of 48 μmol of p-nitrophenylphosphate (pNP)[1] hydrolyzed/min/mg of protein was achieved.

[1] *Abbreviation used in this paper:* pNP, paranitrophenylphosphate.

This preparation of human granulocyte alkaline phosphatase was free of detectable contaminants as judged by immunodiffusion and polyacrylamide gel electrophoresis. The evidence suggests that alkaline phosphatase thus purified is a zinc metalloenzyme and a lipoprotein.

METHODS

Analytical. Cells were counted manually in a hemacytometer. Protein determinations were done by the method of Lowry, Rosebrough, Farr, and Randall, (11) using bovine serum albumin (Armour Pharmaceutical Co., Chicago, Ill.) as a reference standard.

Alkaline phosphatase was assayed with pNP (Sigma Chemical Co., St. Louis, Mo.) as substrate. The assay was performed at 23°C in a Coleman 124 recording spectrophotometer (Coleman Instruments Div., Perkin Elmer Corp., Maywood, Ill.). The reaction mixture consisted of 3 ml of medium, 0.54 mM pNP, 1.0 mM $MgCl_2$, 1.0 M Tris, 0.12 M HCl (pH 9.0) to which 10–200 μl of enzyme was added. Hydrolysis was measured as the change in A_{410} for the first 3 min after mixing, during which time the reaction is linear. A unit of enzyme activity hydrolyzed 1 μmol of pNP/min and was calculated using a measured molar absorptivity of the product of 1.6×10^4 $M^{-1} \cdot cm^{-1}$ under these conditions. Although reaction rates were not linear over large, order-of-magnitude changes in enzyme concentration, they were linear over small (twofold) ones. Hence assays were performed at approximately the same concentrations of enzyme activity, an amount which hydrolyzed 3 nmol of pNP/min/ml.

Preparation of homogenates. Fresh whole blood, drawn in acid citrate dextrose (JA-5, Fenwal Inc., Walter Kidde and Co., Inc., Ashland, Md.), was sedimented for 1 h at unit gravity in an equal volume of a dextran solution consisting of 3.0 g dextran T500 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.), 2.5 g glucose, 0.45 g NaCl and water to 100 ml. The supernatant containing 80% of the leukocytes was harvested, washed, subjected to hypotonic hemolysis, washed, and homogenized in 0.25 M sucrose as previously described (10). Morphology on cells prepared in this fashion was difficult to interpret. Results were therefore expressed in terms of total leukocyte number rather than granulocyte number. The subcellular fractions obtained by differential centrifugation at 400 g consisted of a pellet containing nuclei, unruptured cells, and debris, and a supernatant containing granules, mitochondria, microsomes, and soluble material. The 400 g supernatant was centrifuged at 39,000 g for 30 min. The distribution of alkaline phosphatase between pellet and supernatant under these conditions was not constant. Typical results are shown in Table I.

TABLE I
Fractionation of 400 xg Supernatant of Homogenized Leukocytes at 39,000 xg

	Alkaline phosphatase
	(U/total vol)
S-400	8.82
S-39,000	4.48
P-39,000 extract	25.0

Extraction of alkaline phosphatase from granules. From the layered green and white pellet at 39,000 g, alkaline phosphatase was extracted by adding 2.0 ml of 0.04 M Tris-HCl, pH 8.5, and 4.0 ml of 10 g/100 ml sodium dodecyl sulfate, mixing on a vortex mixer for 60 s once every 10 min, and allowing the suspension to stand for 30 min at 23°C. Insoluble material was removed by centrifugation at 39,000 g. Compared to freeze-thaw, butanol extraction, Triton X-100, and deoxycholate extraction, the described method was found to give the highest yield of enzyme activity in a state from which it could most easily be purified.

Chromatographic procedures. These are described elsewhere (10). Because of a high blank, protein measurements were not made on individual fractions.

Sucrose density gradient centrifugation. Linear gradients from 5 to 20 g/100 ml of sucrose in 0.04 M Tris-HCl, pH 8.5, 0.1% Triton X-100 were established in 5-ml cellulose nitrate tubes. Samples in 0.25 M sucrose were applied to the top in a volume of 0.2 ml. The gradients were centrifuged at 135,000 g at 4° for 16 h in an ultracentrifuge (Beckman Instruments, Inc., Palo Alto, Calif., model L-2) with a SW 50-L rotor. Fractions were collected by pumping from the bottom and visually counting drops. Distance from the top of the gradient was derived by dividing the measured height of each gradient by the number of fractions collected.

Polyacrylamide disk gel electrophoresis. Samples were examined in the system of Davis (12), with various modifications. Best results were obtained using a deoxygenated 7.5% polyacrylamide gel, 0.1 g/100 ml sodium dodecyl sulfate solution, and a Tris-glycine tray buffer at 10 times the concentration described by Davis. The upper tray was made 0.1% in sodium dodecyl sulfate. A 2-h preelectrophoresis at 3 mA/gel was required to remove staining artifacts. Fresh buffer was then added, the samples were applied, and electrophoresis and staining were conducted as previously described (10).

Immunological methods. Rabbits were bled to obtain control sera, and the animals were then immunized with modified Freund's adjuvant suspension containing 10 μg of purified alkaline phosphatase from a polycythemic patient. Initial immunization was in the toe pads. Repeated intramuscular boosting was required to elicit a detectable response. Double-diffusion precipitin analyses (13) were performed in 1.0% Ionagar (Oxoid Division, Consolidated Laboratories, Chicago Heights, Ill.) in 0.04 M Tris-HCl, pH 8.6. Plates were washed on a shaker for 2 h in buffer before staining for enzyme activity, and for 48–72 h in saline and water, and were dried before staining for protein.

Staining procedures. Alkaline phosphatase activity was demonstrated with α-naphthyl acid phosphate and Fast Blue RR (10). Protein was detected with amidoblack, coomassie blue or, in the case of some of the polyacrylamide gels, by UV monitoring of fluorescence produced by reacting 1-dimethylamino-5-naphthalene sulfonyl chloride (dansyl chloride) with the applied samples as described by Talbot and Yphantis (14).

RESULTS

Purification of granulocyte alkaline phosphatases. The sequence of steps used in purification is outlined in Fig. 1. When deoxycholate was substituted for sodium dodecyl sulfate in step 4, the overall yield was reduced by 50–60%, although the results were otherwise comparable to

(1) Dextran sedimentation of whole blood

Upper layer ——————————————————— Lower layer, discard

(2) Hypotonic saline hemolysis; 400 g centrifugation

Pellet ———————————————————— Supernate, discard

(3) Sucrose homogenization (Teflon/glass)
   (a) 400 g centrifugation ———————

Supernate (S-400) ———————————————— Pellet, discard

   (b) 39,000 g centrifugation

Pellet (P-39,000) ———————————————— Supernate, discard

(4) Sodium dodecyl sulfate extraction (6%); 39,000 g centrifugation

Supernate ———————————————————— Pellet, discard

(5) DEAE-cellulose chromatography, 0.04 M Tris, 0.1% Triton X-100, NaCl gradient.
    Desalt active fractions on Sephadex G-25.
(6) Repeat step 5.
(7) Step elution from miniature DEAE-cellulose column, 0.04 M Tris, 0.5 M NaCl, 0.1% Triton X-100.
(8) Sucrose density gradient centrifugation, pool active fractions.
(9) Repeat step 7.

Figure 1 Purification of human granulocyte alkaline phosphatase.

those reported for guinea pig bone marrow alkaline phosphatase (10).

For purposes of comparison, the separate purifications of granulocyte alkaline phosphatase from normal and polycythemic individuals have been presented together. The results of step 5, DEAE-cellulose column chromatography, are presented in the left panel of Fig. 2. The enzyme activity in both cases emerged in broad, widely distributed, nonidentical peaks. The results of rechromatography of all of the activity eluted from the first column on an identical column under identical conditions, step 6, are shown in the right panel of Fig. 2. The majority of the alkaline phosphatase activity was now seen to emerge from each source in a single, symmetrical, identical peak. Both peaks occurred at identical salt strength.

The results of centrifugation of chromatographically purified alkaline phosphatase from step 8 are depicted in Fig. 3. The upper 12 mm of the sucrose gradients contained 54% of the protein. Hence, this step proved useful both as a purification and as an analytical procedure. For comparison purified rabbit IgG was centrifuged under the same conditions. The peak of recovered IgG protein was between 17.2 and 18.6 mm from the meniscus, whereas the peaks of the alkaline phosphatases, as shown in Fig. 3, were between 17.5 and 18.8 mm from the meniscus. After chromatography on Sephadex G-200 (Pharmacia Fine Chemicals, Inc.), however, the alka-

line phosphatases were recovered in the void volume with 60% recovery of enzyme activity.

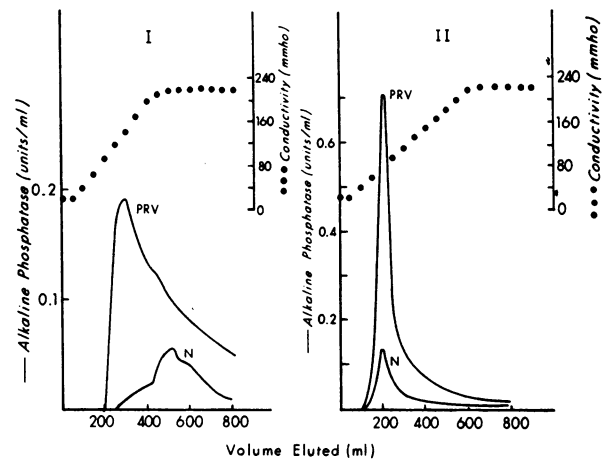The results of a purification using the leukocytes from

Figure 2 Chromatography of alkaline phosphatase on DEAE-cellulose in a linear salt gradient. PRV, polycythemia vera; N, normal. Both gradient were established in 0.04 M Tris, 0.01 M HCl, pH 8.6. (I) Granule extract from step 4, Fig. 1, containing 250 mg (PRV) or 406 mg (N) was applied to 9 g of adsorbent. (II) All recovered activity was pooled, desalted, and chromatographed under identical conditions. Alkaline phosphatase (solid line) and conductivity (dotted line) are plotted as a function of volume.
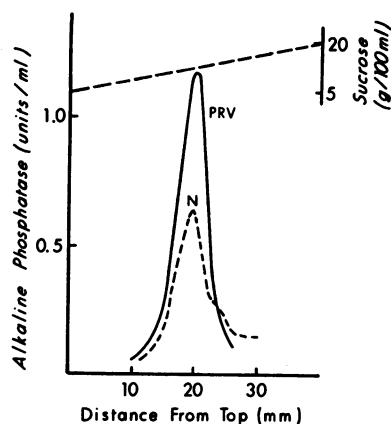
FIGURE 3 Sucrose density gradient sedimentation of PRV and alkaline phosphatase (Step 8). Samples of 0.2 ml were applied to 5-ml sucrose gradients from 5 to 20 g/100 ml in 0.04 M Tris, 0.01 M HCl, 0.1% Triton X-100, pH 8.6 and centrifuged at 135,000 g for 16 h. By means of a siphoning device, 0.2-ml fractions were collected from the bottom of the gradient. Alkaline phosphatase activities are expressed as a function of distance migrated from the top of the gradient. Sucrose gradients were verified by refractometry.

17 U of blood from normal donors are shown in Table II. A 6,910-fold purification was obtained with a 60% yield and a final specific activity of 47 U/mg.

Data from a purification of enzyme from 4 pints of blood from a single polycythemic patient are shown in Table III. Although the purification appears to be six-fold less than that shown in Table II for normal enzyme, the resultant yield and specific activity are comparable.

Polyacrylamide gel electrophoresis. Granulocyte alkaline phosphatases prepared by the means described here were difficult to examine on polyacrylamide gels because they failed to enter the gels in the absence of detergent and failed to stain with standard colored stains. Although various proteins in crude granule extracts made without sodium dodecyl sulfate migrated through polyacrylamide gels under the conditions described by

Davis (12), alkaline phosphatase activity, as demonstrated by histochemical stain and assays on gel slices, did not migrate beyond the first 2 mm even after extensive electrophoresis. Alkaline phosphatases suspended in sodium dodecyl sulfate migrated in a band 6 mm wide, extending from 27 to 23 mm from the top of the 90 mm gel. Under the same electrophoretic conditions, rabbit IgG migrated 33–34 mm into the gel. Gels loaded with purified (step 4) alkaline phosphatase did not stain with either coomassie blue or amidoblack even when 30 μg of protein was applied.

Under the same electrophoretic conditions, dansylated purified (step 4) alkaline phosphatases, inactivated by the boiling procedure required for dansylation (14), migrated 23–26 mm into the gel as indicated by their pattern of fluorescence. Although 0.3 μg or less of protein could be detected by this method, only a single band was seen when 12 μg of protein thus treated was applied to a gel.

Thus, although it was impossible to demonstrate both enzyme activity and protein under identical conditions on polyacrylamide gel, a single band of enzyme activity and a single band of protein with comparable mobilities were demonstrated in purified alkaline phosphatases. Polycythemia vera and normal enzyme behaved identically.

Comparisons and characteristics of purified granulocyte alkaline phosphatases. Values for the amount of alkaline phosphatase activity recovered from the leukocytes of 12 different normal donors and 7 polycythemic patients are shown in Fig. 4. The values for a single polycythemic patient varied by threefold over a 15-mo interval without a concomitant change in leukocyte or granulocyte count. The mean recovery of activity from polycythemic patients was 5.3 times greater than from normal donors.

Purified leukocyte alkaline phosphatase from polycythemic and normal donors had similar affinities for pNP as depicted in Table IV. Similar data were found in the presence and absence of 1.0 mM MgCl₂.

TABLE II
Purification of Granulocyte Alkaline Phosphatase from Normal Subjects

Step	Total protein	Total alkaline phosphatase	Specific activity	Purification	Yield
	mg	*U*	*U/mg*		*%*
1 S-400	2,140	14.7	0.0068	1.0	—
2 SDS-extract	406	31.9	0.079	12.0	100*
3 DEAE-cellulose eluate (Step 7)	7.2	24.0	3.4	470	75
4 Sucrose gradient concentrate	0.128‡	6.0‡	47	6910	60‡

Units are expressed as micromoles of p NP hydrolyzed per minute at 23°C.
* This fraction was chosen to represent 100% activity because of the marked but variable enhancement of activity by the extraction procedure.
‡ Only 42% of the fraction from step 3 was carried through step 4.

TABLE III

Step	Total protein	Total alkaline phosphatase activity	Specific activity	Purification	Yield
	mg	*U*	*U/mg*		*%*
1 S-400	721	44.5	0.06	1.0	—
2 SDS-extract	250	140	0.56	9.0	100*
3 DEAE-cellulose eluate (step 7)	9.4	67.5	7.2	120	49
4 Sucrose gradient concentrate	1.0	70.5	70	1,166	50

U are expressed as micromoles of p NP hydrolyzed per minute at 23°C. The overall degree of purification of this polycythemic patient's alkaline phosphatase is lower than the normal reflecting the greater contribution of alkaline phosphatase to the crude preparation.

* This fraction was chosen to represent 100% activity because of the marked but variable enhancement of activity by the extraction procedure.

Addition of magnesium to the purified alkaline phosphatases enhanced their activity by only 33%, far less than the four- to sevenfold reported for the guinea pig enzyme (10).

Detergent was required in addition to salt to elute alkaline phosphatases from DEAE-cellulose, as shown in Table V. In crude preparations, the maintenance of a

detergent-containing environment was required to recover 95% of the activity applied. Thus, as shown in the first experiment in Table V, if crude enzyme was applied to DEAE-cellulose and washed free of detergent, it could not subsequently be eluted by adding salt to the buffer, or both salt and detergent. However, as shown in experiment 2, if detergent was added to all buffers, keeping the enzyme in a detergent-containing environment, all of the enzyme could be recovered when salt was then added to the buffer. This same behavior held for crude alkaline phosphatases from both normals and patients with polycythemia. As shown in experiment 3, when step 4 enzyme was used (either normal or polycythemia vera), one-third of the enzyme could be recovered without detergent, and the rest of the enzyme could be recovered by adding detergent, although the detergent-containing environment had not been maintained.

Less than 0.004 μg of chloroform-methanol extractable phosphorus was obtained from 40.4 μg of purified alkaline phosphatase protein. Assuming a molecular weight of human leukocyte alkaline phosphatase of 160,000 daltons, this is less than 0.5 mol phosphorus/mol of enzyme.
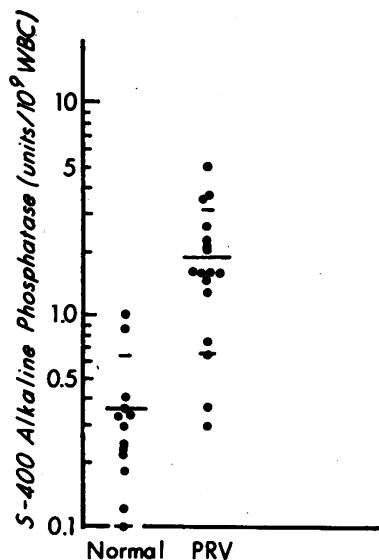

FIGURE 4 Alkaline phosphatase content of leukocyte granules. Values of direct assays on unextracted granule suspensions (S-400 of Fig. 1) are expressed as U/10⁹ leukocytes. Correction for the absolute granulocyte count did not affect the relationships shown. The mean for normals was 0.36 U/10⁹ leukocytes (WBC) and for polycythemics was 1.9 U/10⁹ leukocytes. Assays were done on individual polycythemics on blood obtained at the time of therapeutic phlebotomy. Some of the patients had only a single phlebotomy. Data are expressed as individual determinations (dots) with mean (longer horizontal lines) and standard deviation (shorter horizontal lines).

TABLE IV

K_m for pNP of Purified Granulocyte Alkaline Phosphatases

Source of LAP	K_m	(Range)	N
Patient B. H. polycythemia vera	2.9 × 10⁻⁵	(2.7 − 3.1 × 10⁻⁵)	3
Patient J. H. polycythemia vera	3.3 × 10⁻⁵		1
Normal pool, no. 1	4.0 × 10⁻⁵	(3.9 − 4.1 × 10⁻⁵)	3
Normal pool, no. 2	2.3 × 10⁻⁵	(2.2 − 2.4 × 10⁻⁵)	2

Values for K_m were derived from least mean squares analysis of a double-reciprocal plot (Lineweaver-Burk) of velocity vs. substrate concentration. All determinations were done in duplicate. Conditions of the assay are given in Methods. N is the number of determinations.

TABLE V
Requirement for Detergents to Elute Granulocyte Alkaline
Phosphatase from DEAE-Cellulose*

Source of LAP	Eluting buffers (in sequence)	% Triton	% Recovery
S-400	0.04 M Tris	—	
	0.04 M Tris, 0.2 M NaCl	—	3
	0.04 M Tris, 0.2 M NaCl	0.1	5
S-400	0.04 M Tris	0.1	1
	0.04 M Tris, 0.2 M NaCl	0.1	98
Step 4	0.04 M Tris	—	1
	0.04 M Tris, 0.2 M NaCl	—	34
	0.04 M Tris, 0.2 M NaCl	0.1	75

* Measured amounts of alkaline phosphatase were applied to
0.5-ml columns of DEAE-cellulose, step-eluted sequentially
with the indicated buffer solutions. The effluents were then
assayed for total alkaline phosphatase activity. The results
are expressed as the percentage of activity applied to the ad-
sorbent. The buffer used throughout was 0.04 M Tris, 0.01 M
HCl pH 8.6.

Both forms of highly purified leukocyte alkaline phos-
phatase were inhibited by o-phenanthroline, a chelating
agent which binds Group II-B metals. As shown in Fig.
5, the inhibition of alkaline phosphatase could be ef-
fectively prevented by allowing o-phenanthroline to form
a complex with zinc before adding alkaline phosphatase.

Antibody studies. In Fig. 6, the results of an amido-
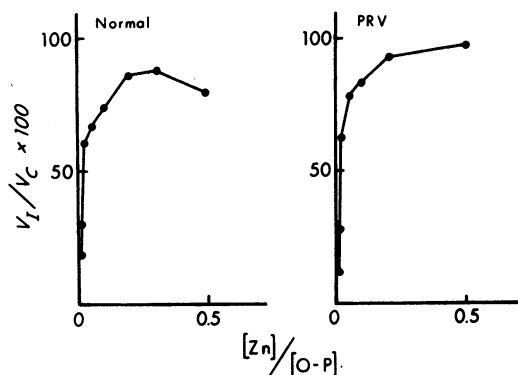blackstained double-diffusion precipitin analysis are

FIGURE 5 Prevention of o-phenanthroline (O-P) inhibi-
tion of alkaline phosphatases by prior addition of zinc. In
the presence of 0.3 mol of zinc/mol of O-P, the reaction
was 88% (normal) and 95% (PRV) of that observed in
the absence of O-P. At higher concentrations of zinc (not
shown) alkaline phosphatase was inhibited. The ratio of
reaction velocity, in the presence (V_i) and absence (V_o) of
O-P is plotted as a function of the ratio of zinc (varied)
to O-P concentration (5×10^{-3} M). Zinc and O-P were
mixed before addition of enzyme. No zinc was added to the
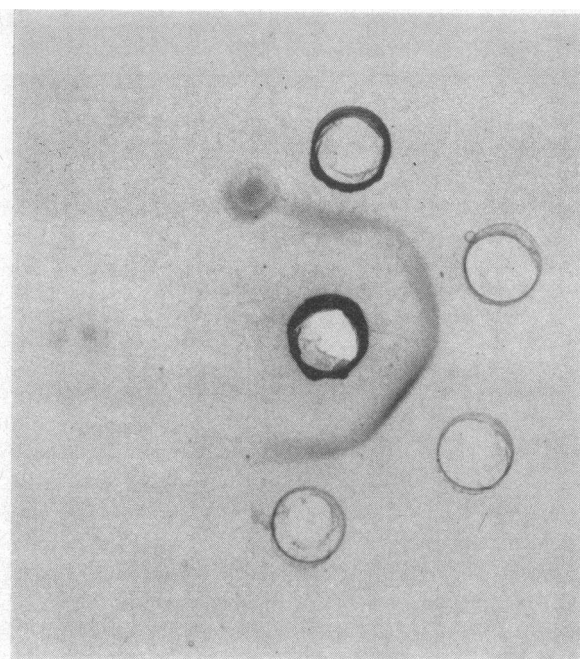control (V_c).

FIGURE 6 Immunologic comparison of normal and polycy-
themia vera granulocyte alkaline phosphatases. The central
well contained antibody to purified step 4 polycythemia
vera granulocyte alkaline phosphatase. The first and third
peripheral wells, clockwise from the top, contained purified
step 4 polycythemia vera granulocyte alkaline phosphatase.
The second and fourth wells contained normal step 4 granu-
locyte alkaline phosphatase. The plate was stained for
protein.

shown. The antiserum, in the central well, was prepared
against step 4 alkaline phosphatase from a polycythemic
patient. The peripheral wells were filled with step 4 alka-
line phosphatases, two from normal pools and two from
polycythemic patients. The principal band demonstrated
showed no evidence of immunologic differences. This
band was also seen by histochemical stain for alkaline
phosphatase activity. A second minor band was present
in one of the normal preparations.

DISCUSSION

Detergent extraction of human leukocyte granules re-
sulted in greater recovery of alkaline phosphatase ac-
tivity and greater ease in manipulation than other meth-
ods tested. This finding was in concordance with the data
reported for the guinea pig leukocyte granule alkaline
phosphatase (10). With the purification procedures de-
scribed here, 50–60% of the enzyme was recovered with
a specific activity of 47–70 μmol of pNP hydrolyzed/min/
mg of protein at 23°C. Organic extraction methods have
been reported to result in 26% overall recovery with a
specific activity of 13 μmol of pNP hydrolyzed/min/mg
of protein (assuming a nitrogen content of 16%) at
37°C (15).

An additional objective in the purification of this granule enzyme was the avoidance of disruption of intramolecular bonds, such as lipid-protein bonds. Evidence has been previously presented (10) that guinea pig leukocyte alkaline phosphatase is a lipoprotein containing 2–4 µg of organically extractable phosphorus/mg of protein when purified using detergents. Although similar methods were used, there was no measurable phosphorus demonstrated (i.e., less than 0.1 µg/mg of protein) in organic extracts of purified human granulocyte alkaline phosphatase. However, the requirement for detergent in the elution of purified alkaline phosphatase from DEAE-cellulose suggests that this procedure permitted the enzyme to retain some of its lipophilic properties although it may have a very low phosphorus content.

The principal objective of these studies was to find chemical evidence, if any exists, for a difference between granulocyte alkaline phosphatase from normal subjects and polycythemic patients. The data show no significant differences in the course of purification or in the final purified product with respect to chromatographic properties or final specific activities. Although the elution pattern varied widely when crude alkaline phosphatases were chromatographed on DEAE-cellulose, this appeared to be a consequence of lipophilic interactions. The entire pool of eluted activity, representing 100% of the activity applied, eluted in a much more homogeneous pattern when rechromatographed under identical conditions, and no significant differences in behavior were observed on the second DEAE-cellulose chromatography in any of the preparations. Substrate affinities and kinetic properties were not significantly different. Enzymes from both sources behaved like zinc metalloenzymes when exposed to the group II-B specific chelating agent, o-phenanthroline. This behavior would be expected because of previous work which has demonstrated that human leukocyte alkaline phosphatase (15) is a zinc metalloenzyme. Finally, both enzymes appeared to be immunologically identical.

We have repeatedly demonstrated only a single species of alkaline phosphatase in these preparations on sedimentation in sucrose gradients and polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, and our findings would thus appear to be in conflict with reports of separable entities on starch gel electrophoresis (16–18). It should be emphasized, however, that the studies reported here dealt with a subcellular fraction of granules, rather than with whole leukocyte extracts, and with enzyme solubilized in sodium dodecyl sulfate rather than an organic solvent extract. Lastly, we have noticed a tendency for a portion of the enzyme to aggregate and precipitate under various conditions such as chilling.

The failure to find chemical differences between the granulocyte alkaline phosphatases from normal and polycythemic subjects does not establish their absence. Rather, by analogy to proteins studied in detail, such as hemoglobin, single amino acid differences, for example, may exist between the enzyme isolated from different individuals. The observations reported shed no light on structural differences that may exist within intact granules.

The evidence does support the hypothesis that the observed difference in alkaline phosphatase activity between the leukocytes of normal subjects and patients with polycythemia vera is a reflection of the quantity of enzyme synthesized, rather than some structural difference. The finding that 3–10 times more enzyme could be extracted from the granules of polycythemic patients than from the granules of normal subjects suggests that this is the case. Observations lending further support to this hypothesis are the similar final specific activities, kinetic data, chromatographic behavior, and apparent molecular size. The failure to observe an immunologic difference between normal and polycythemic alkaline phosphatase also permits an interpretation of identity. It is possible to account for the difference between normal and polycythemic leukocyte granule alkaline phosphatase activity by differences in the quantity of enzyme contained in the granules.

The etiology of polycythemia vera and the mechanisms controlling the formation of blood cells in this disease are poorly understood. Is the disordered granulocyte formation reflected in a marked increase in granule alkaline phosphatase production? If so, understanding this intracellular process may help to explain the change in regulation of granulocyte maturation. The availability of purified granulocyte alkaline phosphatase and antibody to it may facilitate studies of these processes.

Studies are currently being extended to the leukocyte alkaline phosphatases from patients with other diseases, such as chronic myelogenous leukemia, and will be the subject of a later report.

ACKNOWLEDGMENTS

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