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Purification and Characterization of a Phagocytosis-Stimulating Factor from Phagocytosing Polymorphonuclear Neutrophils: Comparison with Granule Basic Proteins

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Phagocytosis-stimulating factor (PSF) was purified by copper chelate chromatography and characterized in comparison with basic proteins in the granule of polymorphonuclear neutrophils. By copper chelate chromatography, PSF was eluted at pH 3.7; whereas cationic protein, lysozyme, and lactoferrin were eluted at pH 5.6, 5.1, and 4.0, respectively. Purified PSF has an approximate molecular weight of 16,000 and an isoelectric point at 8.7, which differ from those of basic proteins, such as cationic protein, lysozyme, and lactoferrin. Anionic substances such as DNA and heparin did not influence the phagocytosis-stimulating activity of PSF, whereas that of the granule basic protein fraction from resting polymorphonuclear neutrophils was abolished. PSF had little bactericidal activity against *Escherichia coli* and *Staphylococcus aureus*, whereas the granule basic protein fraction from resting PMNs had strong bactericidal activity against *E. coli* and weak activity against *S. aureus*. These results indicate that PSF is a basic protein which is distinguishable from cationic protein, lysozyme, and lactoferrin.

Phagocytosis and the killing of invading microorganisms are the most important contribution of polymorphonuclear neutrophils (PMNs) to the host defense. Previously, we have reported that phagocytosis-stimulating factor (PSF), which is generated in the granule fraction of PMNs during phagocytosis (5), enhances the ingestion step of C3b receptor-mediated phagocytosis by PMNs specifically (6). Recently, it has been reported that cationic protein and lysozyme, a component in the granule of PMNs, can enhance the phagocytic activity of PMNs (14, 16). These findings suggest that PSF may be identical to one of the already known basic proteins in the granule. Therefore, we attempted to purify and characterize the PSF and compare it with known basic proteins.

MATERIALS AND METHODS

Animals. Hartley guinea pigs (weight, ca. 500 g) were used as the source of PMNs.

Reagents. Glycogen, zymosan A, and sodium borohydride were obtained from Sigma Chemical Co., St. Louis, Mo. 1,4-Butanediol diglycidyl ether and imminodiacetic acid disodium salt were obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis. Sephadex G-100, Sepharose CL-4B, and a low-molecular-weight calibration kit were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Ampholyte (pH range 3.5 to 10.0) was obtained from LKB Produkter, Bromma, Sweden. An isoelectric point marker protein kit was obtained from Oriental Yeast Co., Ltd. Osaka, Japan.

Preparation of PMNs. PMNs were isolated from the peritoneal cavity 13 to 15 h after intraperitoneal injection of sterilized 0.12% glycogen in 0.9% saline as described previously (26). The collected cells, which contained >96% neutrophils, were suspended at 2×10^7 cells per ml in Hanks balanced salt solution (HBSS).

Opsonization of zymosan. Zymosan particles were opsonized with homologous fresh serum at 37°C for 30 min as described previously (5).

Preparation of partially purified PSF. Partially purified PSF was prepared from phagocytosing PMNs as described previously (6). A total of 10^7 PMNs per ml in HBSS were incubated with 1.0 mg of opsonized zymosan particles per ml at 37°C for 60 min with constant shaking, washed once by centrifugation at $100 \times g$ for 10 min, and suspended in phosphate-buffered saline (PBS) to one-tenth of the original volume. Then, phagocytosing PMNs were disrupted by sonication for 2 min on ice with intermittent pulses at 168 W (Supersonic vibrator, model UR-150 P; Tominaga Works Ltd.) and centrifuged at $100,000 \times g$ for 60 min at 4°C. The resulting crude supernatant containing PSF was heated at 80°C for 30 min and then centrifuged at $100,000 \times g$ for 60 min at 4°C to remove the denatured materials. No PSF activity was detected in the extract prepared from opsonized zymosan suspension without PMNs in the same manner, indicating that PSF is not derived from opsonized zymosan. PSF was chromatographed over a Sephadex G-100 column in PBS (pH 7.2) at 4°C. PSF fractions corresponding to approximately 16,000 daltons were collected, dialyzed against distilled water, and stored at −60°C after lyophilization. Lyophilized PSF was dissolved in PBS before use.

Preparation of granule basic protein fraction. Resting or phagocytosing PMNs, prepared as described above, were suspended in 0.34 M sucrose at a concentration of 8×10^7 cells per ml and homogenized at 0°C in a Teflon-glass homogenizer. A granule-rich fraction was obtained by differential centrifugation of the homogenates as previously described (5). To characterize the granule-rich fractions obtained from resting and phagocytosing PMNs, the subcellular distribution of various enzyme activities in both PMNs was examined. β-Glucuronidase, lysozyme, and lactate dehydrogenase activities were assayed as described previously (23). β-Glucuronidase and lysozyme activities were predominantly present in the granule fraction of resting PMNs, whereas remarkable loss of these enzymes was observed in the granule-rich fraction from phagocytosing PMNs without changes of enzyme distribution in other fractions, presumably because of the degranulation during phagocytosis (8, 25).

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TABLE 1. Subcellular distribution of various enzyme activities in PMNs

Cell	Fraction	Enzyme activity[a]		
		β-Glucuronidase	Lysozyme	LDH
Resting	Nuclei and debris	5.11 ± 0.98	2.04 ± 0.33	0.040 ± 0.030
	Granule	9.99 ± 0.66	3.70 ± 0.14	0.013 ± 0.008
	Postgranule	2.25 ± 0.29	0.09 ± 0.01	0.653 ± 0.193
Phagocytosing	Nuclei and debris	6.62 ± 1.19	1.75 ± 0.34	0.028 ± 0.003
	Granule	4.20 ± 0.17	1.15 ± 0.54	0.030 ± 0.005
	Postgranule	3.16 ± 0.11	0.02 ± 0.01	0.813 ± 0.018

[a] β-Glucuronidase, lysozyme, and lactatedehydrogenase (LDH) activities are expressed as micrograms of phenolphthalein per hour per 10^7 PMNs, micrograms of egg white lysozyme per 10^7 PMNs, and the change in optical density at 340 nm per minute per 10^7 PMNs, respectively. The values represent the means ± standard errors of three experiments.

Each granule-rich fraction obtained was suspended in 0.01 M citric acid at a concentration equivalent to 2.5×10^8 cells per ml, stirred for 60 min at 0°C, and then centrifuged at 100,000 × *g* for 60 min by the method of Lehrer et al. (9). The resulting supernatant is referred to as the granule basic protein fraction. When used for phagocytosis assay or bactericidal assay, the granule basic protein fraction from resting PMNs was dialyzed against PBS.

Copper chelate affinity chromatography. To perform the copper chelate affinity chromatography, epoxy-activated Sepharose CL-4B was prepared by the method of Sundberg and Porath (22). To 4 g of suction-dried Sepharose CL-4B was added 4 ml of 1,4-butanediol diglycidyl ether and 4 ml of 0.6 N NaOH containing 2 mg of $NaBH_4$ per ml. The mixture was rotated at 25°C for 8 h and then washed with distilled water. Subsequently, biscarboxymethylamino-Sepharose CL-4B was prepared by the method of Porath et al. (15). To 2 ml of suction-dried epoxy-activated Sepharose CL-4B was added 0.54 g of imminodiacetic acid disodium salt dissolved in 2.66 ml of 2 M Na_2CO_3, incubated at 65°C for 24 h, and then washed with distilled water.

Copper chelate chromatography was performed by the method of Torres et al. (24). In brief, biscarboxymethylamino-Sepharose CL-4B was packed into a column (5.5 by 140 mm) and then 3 ml of $CuSO_4$ solution (5 mg/ml) was pumped in at a rate of 5 ml/h, followed by the injection of 30 ml of distilled water. The column was equilibrated with 40 mM Tris–5 mM phosphate–0.5 M NaCl buffer (pH 8.2). Granule basic protein fraction from resting or phagocytosing PMNs was mixed with one-half volume of 40 mM Tris–5 mM phosphate–0.5 M NaCl buffer (pH 8.2), adjusted to pH 8.2 with 0.1 N NaOH, and then centrifuged at 1,870 × *g* for 10 min to remove precipitates. This sample was applied to the column and then eluted with a 100-ml linear gradient from sodium phosphate–0.5 M NaCl (pH 7.7) to 0.1 M acetic acid–0.5 M NaCl (pH 2.8) at a flow rate of 5 ml/h, and fractions of 2.0 ml were collected. Pooled fractions were dialyzed against distilled water, lyophilized, stored at −60°C, and dissolved in PBS to the original sample volume before use.

Polyacrylamide gel electrophoresis. Sodium dodecyl sulfate (SDS)-polyacrylamide slab gel electrophoresis was carried out with 15% polyacrylamide in 1% SDS by the method of Laemmli and Favre (7) with slight modification (4), and native polyacrylamide gel electrophoresis was performed by the method of Reisfeld et al. (17) with slight modification (24). The gel was stained with Coomassie brilliant blue R-250.

Isoelectric focusing. Isoelectric focusing was carried out on polyacrylamide disc gels (1 by 60 mm), containing ampholytes in the pH range of 3.5 to 10.0, by the method of Manabe et al. (10). Electrode solutions employed were 10 mM H_3PO_4 as anode electrode solution and 40 mM NaOH as cathode electrode solution. Isoelectric focusing was performed at 0.1 mA of constant current per tube for 30 min and then at 300 V of constant voltage for 2 h. The gel was stained with Coomassie brilliant blue R-250.

Amino acid analysis. Estimation of the amino aid composition of PSF was made with samples treated with 6 N HCl at 110°C for 24 h in evacuated sealed tubes. The analysis was performed with an Hitachi 835 high-speed amino acid analyzer (11).

Assay for phagocytosis. The phagocytic activity of PMNs was assayed as described previously (6). In brief, a 0.4-ml

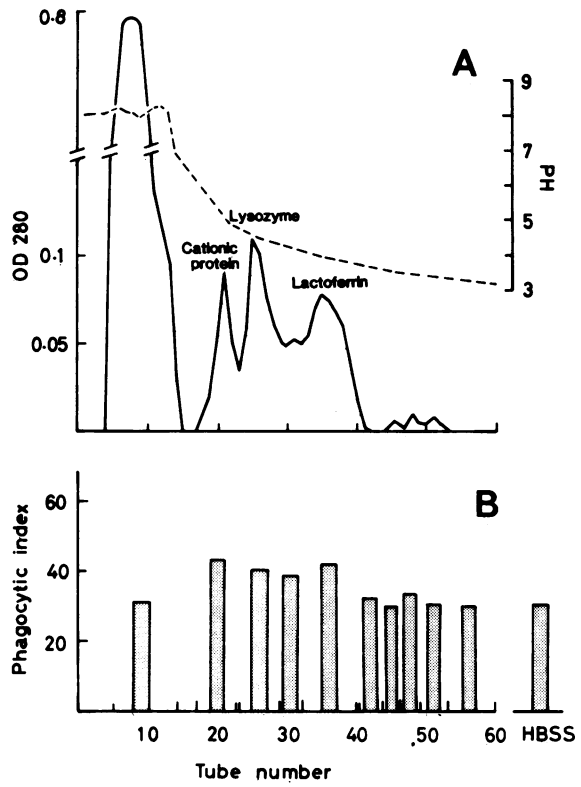

FIG. 1. Copper chelate chromatography of granule basic protein fraction from resting PMNs. (A) The column (5.5 by 140 cm) was equilibrated with 50 mM Tris–5 mM phosphate–0.5 M NaCl buffer (pH 8.2), and the sample (1.37 mg of protein; 3.0 ml) was applied to the column. Elution was carried out with a 100-ml linear gradient from 20 mM sodium phosphate–0.5 M NaCl (pH 7.7) to 0.1 M acetate–0.5 M NaCl (pH 2.8) at a flow rate of 5 ml/h. Fractions of 2.0 ml were collected and assayed for the absorbance at 280 nm (OD 280; ——) and pH values (– – –). Pooled fractions were dialyzed against distilled water, lyophilized, and then dissolved in PBS at the original sample volume. (B) Phagocytosis-stimulating activity of each fraction was determined. PMN monolayers (4×10^5 cells per dish) were incubated at 37°C for 10 min with 2.4×10^6 opsonized zymosan particles in the presence of 400 μl of each fraction or HBSS as the control. Phagocytosis was assessed microscopically. The figure is representative of three separate experiments with similar results. Phagocytosis-stimulating activity is expressed as the mean value of duplicate assays.

sample of PMN suspension (10^6 cells per ml) in HBSS supplemented with 20% heat-inactivated guinea pig serum was placed into a 35-mm plastic petri dish containing two glass cover slips (22 by 10 mm). Cells were permitted to adhere to the cover slips for 1 h at 37°C and then washed twice with HBSS to remove nonadhering cells. Phagocytosis was initiated by the addition of 0.24 ml of opsonized zymosan suspension (10^7 particles per ml) to the PMN monolayer in a petri dish with or without test samples in a total volume of 2.0 ml. After incubation at 37°C for 10 min, the cover slips were washed with HBSS, fixed with ethanol, and stained with Wright-Giemsa; and phagocytosis was determined microscopically. The phagocytic index was defined as the percentage of positive ingestion multiplied by the average number of ingested particles per cell.

Assay for bactericidal activity. *Escherichia coli* NIHJ-2 and *Staphylococcus aureus* NIHJ-1 were used for the bactericidal assay. Each species of bacteria was cultured in nutrient broth for 14 to 16 h at 37°C and then sedimented by centrifugation at 6,000 × *g* for 10 min, washed twice, and suspended in sterile PBS (pH 6.5). Microbial concentrations were determined turbidimetrically by measuring absorbance at 650 nm (20). To 0.05 ml of bacterial suspension (10^4 CFU/ml) was added various concentration of test samples in a total volume of 0.5 ml of PBS (pH 6.5) and incubated for 2 h at 37°C. After incubation a fraction (0.1 ml) was plated on nutrient agar, and the resulting colonies were counted in 18 to 24 h. Results were expressed as the percentage of viable bacteria to the percentage of control bacteria.

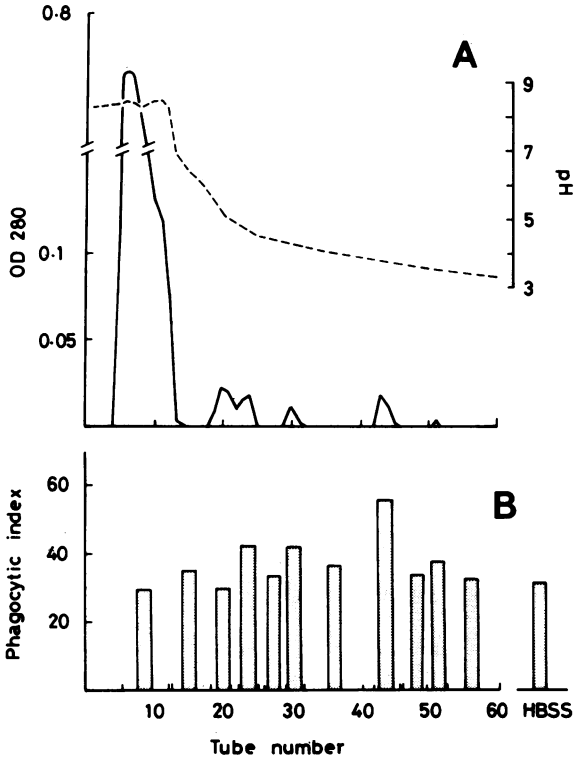

FIG. 2. Copper chelate chromatography of granule basic protein fraction from phagocytosing PMNs. (A) The sample (0.632 mg of protein; 2.25 ml) was applied to the column, and elution was carried out as described in the legend to Fig. 1. (B) Each fraction (400 μl) was tested for phagocytosis-stimulating activity. The figure is representative of three separate experiments with similar results. Phagocytosis-stimulating activity is expressed as the mean value of duplicate assays.

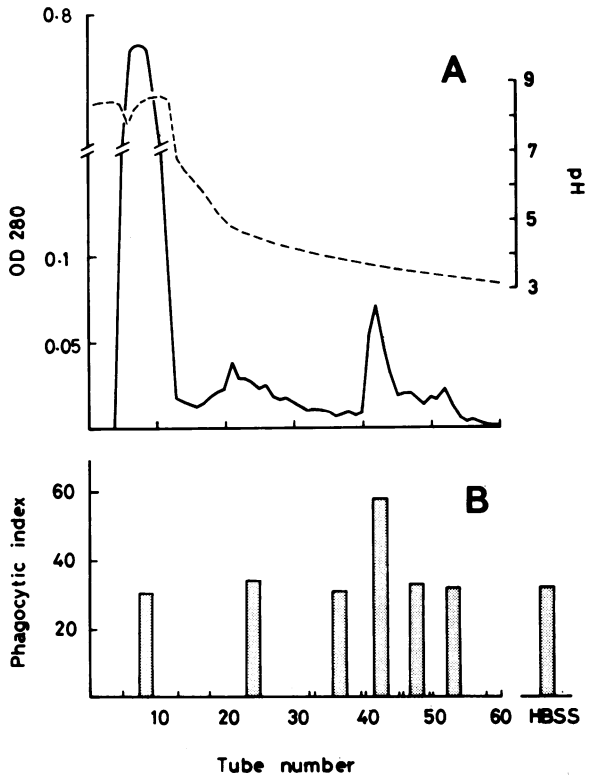

FIG. 3. Copper chelate chromatography of partially purified PSF. (A) Partially purified PSF (3.12 mg of protein; 2.7 ml) was applied to the column and eluted through a pH gradient. (B) Each fraction (400 μl) was tested for phagocytosis-stimulating activity as described in the legend to Fig. 1. The figure is representative of three separate experiments with similar results. Phagocytosis-stimulating activity is expressed as the mean value of duplicate assays.

RESULTS

Copper chelate chromatography of granule basic proteins and PSF. Purification of partially purified PSF obtained by chromatography on Sephadex G-100 was attempted on a DEAE cellulose column equilibrated with 10 mM Tris-hydrochloride buffer (pH 8.5). However, PSF activity was eluted to the nonadsorbed fraction, indicating that PSF is basic.

It has been reported that copper chelate affinity chromatography is useful for separating basic proteins in the granule fraction of PMNs (13, 24). Thus, we examined whether copper chelate chromatography was useful for purification of PSF. Figure 1 shows the results of copper chelate chromatography of granule basic protein fraction from resting PMNs. The decreasing pH gradient resulted in the elution of cationic protein, lysozyme, and lactoferrin at pH 5.6, 5.1, 4.0, respectively, which was compatible with the results of Torres et al. (24). Lysozyme and lactoferrin were identified by polyacrylamide gel electrophoresis by the method of Reisfeld et al. (17), with hen egg white lysozyme and human milk lactoferrin used as standards. The cationic protein obtained was identified by its more rapid migration toward the cathode than that observed for lysozyme on this electrophoretogram, as reported by Torres et al. (24). Each fraction slightly enhanced phagocytosis by PMNs as com-

TABLE 2. Purification of PSF

Step	Total protein (mg)	Total activity (U)[a]	Sp act (U/mg)	Purification (fold)	Yield (%)
1. Cell lysate	685.4	ND[b]			
2. 10,000 × g supernatant	267.3	3,873.9	14.49	1	100
3. Heat-treated (80°C, 30 min) supernatant	111.9	3,291.2	29.41	2.0	85.0
4. Chromatography on Sephadex G-100	3.12	2,080.0	666.67	46.1	53.7
5. Chromatography on copper chelate	0.44	1,333.3	3,030.30	209.1	34.4

[a] One unit of PSF activity is defined as the amount which in 1 ml causes 50% of the maximal phagocytosis-stimulating response.
[b] ND, Not determined.

pared with control, which was supported by the results of a previous report that states that granule cationic protein and lysozyme are able to enhance the phagocytic activity of PMNs (16). The other small protein peaks that were eluted at pHs below 3.5 did not have phagocytosis-stimulating activity.

However, when granule basic protein fraction from phagocytosing PMNs was chromatographed on a copper chelate affinity column (Fig. 2), an extremely different elution pattern was observed. The peaks corresponding to cationic protein, lysozyme, and lactoferrin diminished in this chromatograph, presumably because of the degranulation during phagocytosis (8, 25). Another noticeable difference was the appearance of a new peak at pH 3.7 which possessed strong phagocytosis-stimulating activity (phagocytic index, 56.0). This protein peak, which had an apparent molecular weight of 16,000 as estimated by SDS-polyacrylamide gel electrophoresis, has not been observed in the case of chromatography of the granule basic protein fraction from resting PMNs (Fig. 1). These results indicate that PSF can be separated from cationic protein, lysozyme, and lactoferrin by copper chelate chromatography and support results of our earlier report that PSF is generated in the granule fraction of PMNs during phagocytosis (5). Therefore, we used this procedure for the purification of PSF.

When partially purified PSF was chromatographed on a copper chelate affinity column (Fig. 3), phagocytosis-stimulating activity was recovered at pH 3.7. No significant phagocytosis-stimulating activity was found in other fractions. In this chromatograph, 95% of the protein applied to the column was recovered. The contents of cationic protein, lysozyme, and lactoferrin in partially purified PSF were 1.4, 2.4, and 1.7% protein, respectively, as determined by their recoveries on copper chelate chromatography, but no myeloperoxidase activity was detected. A summary of the purification procedure for PSF is given in Table 2. The final amount of PSF thus obtained from 1.14×10^{10} phagocytosing PMNs (685.4 mg of protein) was 0.44 mg.

SDS-polyacrylamide gel electrophoresis of PSF obtained by copper chelate chromatography. PSF obtained by copper chelate chromatography proved to be a single protein band with an apparent molecular weight of 16,000, indicating that PSF was completely purified by copper chelate chromatography (Fig. 4). However, cationic protein, lysozyme, and lactoferrin were observed to have molecular weights of 36,000, 14,500, and 70,000, respectively, on SDS-polyacrylamide gel electrophoresis, indicating the distinction between PSF and granule basic proteins on the basis of molecular weights.

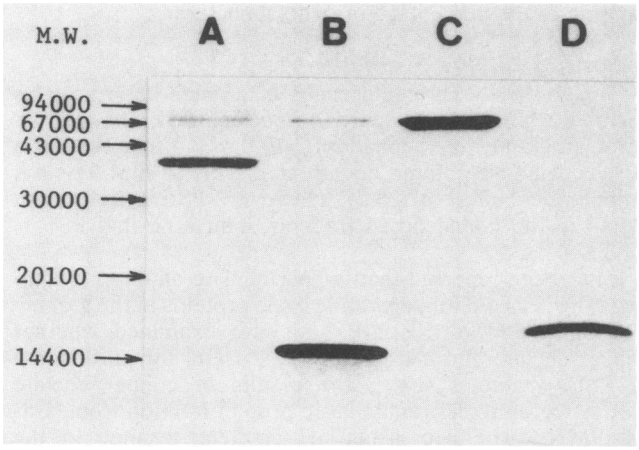

FIG. 4. SDS-polyacrylamide gel electrophoresis of purified PSF and granule basic proteins. Polyacrylamide gel electrophoresis was carried out with 15% polyacrylamide in 1% SDS. Each sample obtained by copper chelate chromatography was applied to SDS-polyacrylamide gels. Lane A, 4 μg of cationic protein; lane B, 3 μg of lysozyme; lane C, 6 μg of lactoferrin; lane D, 2 μg of purified PSF. Protein standards (molecular weights [m.w.]) employed were phospholipase b (94,000), albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and α-lactoalbumin (14,400).

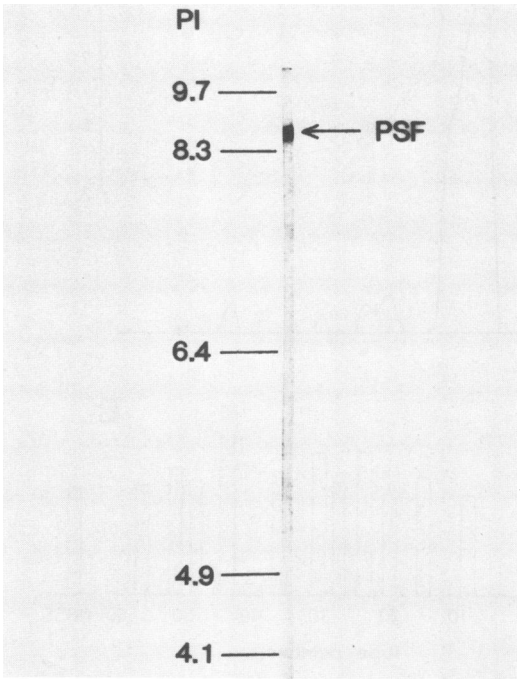

FIG. 5. Isoelectric focusing of PSF. Purified PSF (2 μg of protein) was applied on top of the gel column, and isoelectric focusing was performed at 0.1 mA of constant current per tube for 30 min and then at 300 V of constant voltage for 2 h. The gel was stained with Coomassie brilliant blue R-250. Cytochrome *c* at pIs of 10.6, 9.7, 8.3, 6.4, and 4.9, and 4.1, acetylated to various degrees, was employed as pI markers.

TABLE 3. Amino acid composition of PSF[a]

Amino acid	Percentage of total	No. of residues per molecule of PSF[b]	
		Actual	Nearest integer
Aspartic acid	13.1	18.6	19
Threonine	5.2	7.3	7
Serine	7.1	10.1	10
Glutamic acid	15.8	22.4	22
Proline	2.3	3.3	3
Glycine	8.1	11.4	11
Alanine	5.3	7.6	8
Half-cystine	1.0	1.4	1
Valine	2.0	2.8	3
Methionine	2.0	3.2	3
Isoleucine	4.3	6.2	6
Leucine	9.9	14.0	14
Tyrosine	1.1	1.6	2
Phenylalanine	4.1	5.8	6
Histidine	7.7	10.9	11
Lysine	7.2	10.3	10
Arginine	4.0	5.7	6

[a] Purified PSF (100 μg of protein) obtained by copper chelate chromatography was hydrolyzed with 6 N HCl in an evacuated sealed tube at 110°C for 24 h.

[b] Based on a molecular weight of 16,000 determined by SDS-polyacrylamide gel electrophoresis. There was a total of 142 residues.

Isoelectric focusing electrophoresis of purified PSF. Isoelectric focusing electrophoresis of purified PSF showed one protein band with an apparent isoelectric point at 8.7 (Fig. 5). This result indicates that PSF is a basic protein.

Amino acid analysis of purified PSF. The amino acid composition of the purified PSF is shown in Table 3. The acidic amino acid residues outnumber the basic ones, suggesting that acidic amino acids exist in the amide form.

Effect of anionic substances on phagocytosis-stimulating activity of PSF. It has been reported that antibacterial activity and phagocytosis-enhancing activity of cationic protein are abolished by various anionic substances, such as DNA, heparin, or endotoxins (1, 16, 19, 28). Therefore, we examined the effect of DNA and heparin on the phagocytosis-stimulating activity of PSF in comparison with the granule basic protein fraction from resting PMNs. The phagocytosis-stimulating activity of PSF reached a plateau at a concentration of 5.0 μg/ml, whereas that of the granule basic protein fraction from resting PMNs reached a plateau at a concentration of 10.0 μg/ml, although its activity was less than that of PSF. From these results, the effect of anionic substances on the phagocytosis-stimulating activity of PSF and the granule basic protein fraction from resting PMNs was examined at a concentration of 5.0 μg/ml for PSF and 10.0 μg/ml for the granule basic protein fraction from resting PMNs. The phagocytosis-enhancing activity of PSF was not significantly reduced by heparin or DNA, whereas that of the granule basic protein fraction from resting PMNs was inhibited by heparin or DNA in a dose-dependent manner and completely abolished by 10 μg of heparin or 25 μg of DNA per ml (Fig. 6). Heparin and DNA (25 μg/ml each) did not influence phagocytosis by PMNs (data not shown).

Bactericidal activity of PSF. It has been reported that a number of basic proteins have antimicrobial activity (9, 12, 27). Thus, we examined the effect of PSF on *E. coli* and *S. aureus*. PSF exhibited little bactericidal activity against both *E. coli* and *S. aureus*, whereas the granule basic protein fraction from resting PMNs displayed the bactericidal activity against *E. coli* in a dose-dependent manner but lesser activity against *S. aureus*, with 86% of *E. coli* being killed by 40 μg of granule basic protein fraction from resting PMNs per ml (Fig. 7).

DISCUSSION

We have reported previously that PSF is generated in the granule fraction of phagocytosing PMNs (5). It is well known that granules of PMNs contain various basic proteins, such as cationic protein, lysozyme, and lactoferrin, which have bactericidal or bacteriostatic activity (12, 21, 27). Recently, Pruzanski and Saito (16) have demonstrated that granule

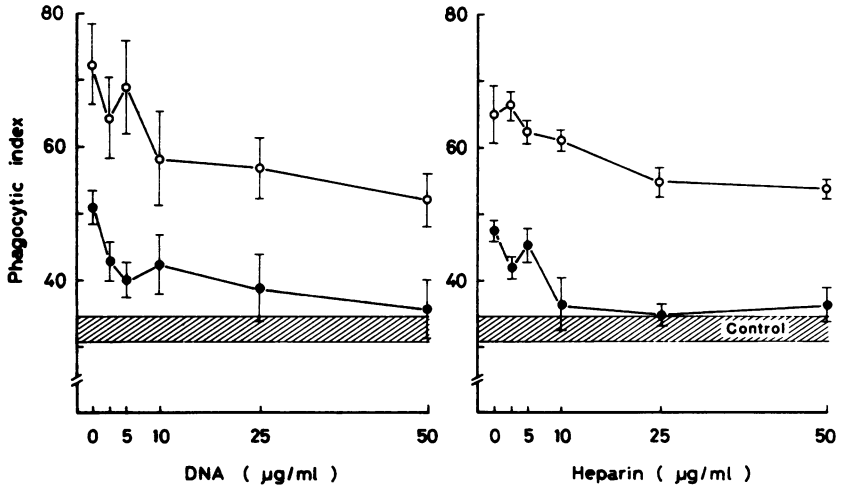

FIG. 6. Effect of anionic substances on phagocytosis-stimulating activity of PSF and the granule basic protein fraction. PMN monolayers (4×10^5 cells per dish) were incubated with 2.4×10^6 opsonized zymosan particles in the presence of various concentrations of DNA or heparin in a total volume of 2.0 ml of HBSS containing 5 μg of partially purified PSF per ml (○) or 10 μg of granule basic protein fraction per ml (●) at 37°C for 10 min. After incubation, phagocytosis was assayed microscopically. The control range of phagocytosis is shown in the shaded areas. The values represent the means ± standard errors of three experiments. Each phagocytosis-stimulating activity of PSF (5 μg/ml) and the granule basic protein fraction from resting PMNs (10 μg/ml) in the absence of DNA or heparin was significant ($P < 0.01$), as judged by Student's t test.

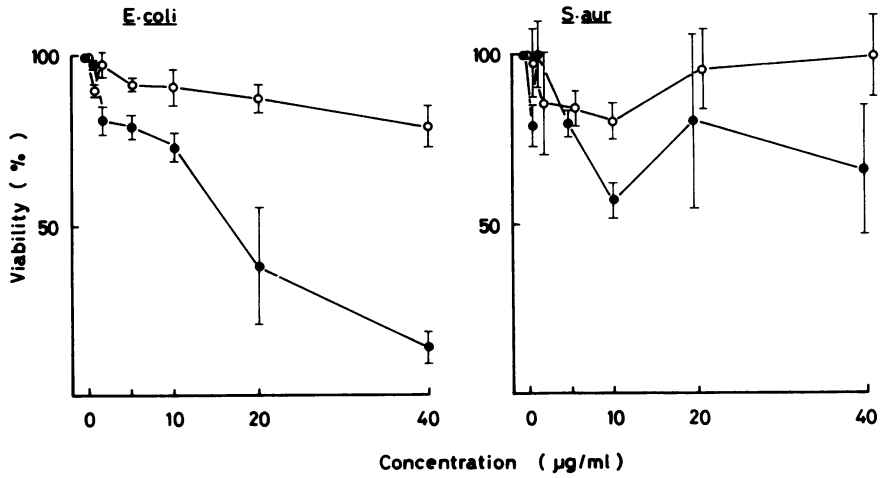

FIG. 7. Bactericidal activity of PSF and granule basic protein fraction. *E. coli* or *S. aureus* (S. aur) (5×10^2 bacteria) was incubated with various concentrations of partially purified PSF (○) or granule basic protein fraction (●) in a total volume of 0.5 ml of PBS (pH 6.5) at 37°C for 2 h. The viability of bacteria was determined by colony count. Values represent the means ± standard errors of three experiments.

cationic protein and lysozyme, as well as synthetic cationic substances, enhance the phagocytic activity of PMNs. Therefore, in the present study, we attempted to purify PSF by copper chelate chromatography and characterize it in comparison with the granule basic proteins of PMNs.

On copper chelate chromatography, PSF was eluted at pH 3.7 (Fig. 2 and 3), whereas cationic protein, lysozyme, and lactoferrin were eluted at pH 5.6, 5.1, and 4.0, respectively (Fig. 1), suggesting that PSF is different from cationic protein, lysozyme, and lactoferrin. Results of SDS-polyacrylamide gel electrophoresis also indicate that PSF is clearly distinct from cationic protein and lactoferrin, based on molecular weights (Fig. 4). Although the molecular weight of lysozyme closely resembled that of PSF, the isoelectric points were quite different from each other; i.e., PSF has an isoelectric point at 8.7 (Fig. 5), whereas lysozyme has an isoelectric point at 10.0 to 11.0 (18). PSF obtained from partially purified PSF seems to be identical to that in the granule basic protein fraction from phagocytosing PMNs, based on the elution profile on copper chelate chromatography and molecular weights (Fig. 2 through 4).

It has been reported that the granule cationic protein of guinea pig PMNs contains a large amount of basic amino acids, particularly arginine (16%) (27), and that histones, which are basic proteins in nuclei, are rich in alanine (11 to 27%) and lysine (11 to 34%), as well as arginine (7 to 15%) (2, 3). However, PSF is basic (pI 8.7) without a sufficiently high basic amino acid content, only 4% arginine and 7.2% lysine in total amino acids (Table 3), and with a high amount of acidic amino acids, suggesting that acidic amino acids are in the amide form. These findings indicate that PSF is distinguishable from cationic protein and histone.

It has been reported that anionic substances such as heparin, RNA, and DNA can abolish the phagocytosis-enhancing activity of cationic protein, lysozyme, and synthetic cationic substances (16). In our studies, the phagocytosis-stimulating activity of PSF was influenced only slightly by DNA and heparin (Fig. 6). Because PSF is not as basic as the basic proteins described above, anionic substances did not seem to interact electrostatically with PSF efficiently to abolish its phagocytosis-enhancing activity under our experimental conditions.

Because granule basic proteins have the bactericidal or bacteriostatic activities and contribute to nonoxidative microbial killing mechanisms of PMNs (9, 12, 27), it is of interest to test whether PSF has bactericidal activity. However, neither *E. coli* nor *S. aureus* was susceptible to PSF treatment (Fig. 7), suggesting that PSF may not be involved in the bacterial killing mechanisms of PMNs directly. Although partially purified PSF contained only 5.5% granule basic proteins such as cationic protein, lysozyme, and lactoferrin, such small amounts of granule basic proteins would not affect the assay for the bactericidal activity.

Results of this study indicate that PSF is a new basic protein which is distinguishable from any known basic proteins, based on chromatographic, physicochemical, and biological properties. The mechanisms by which PSF is generated from PMNs during phagocytosis are still uncertain. However, it has been assumed that the generation of PSF would not result from de novo synthesis but from the activation of a PSF precursor because of no effect of cycloheximide, an inhibitor of protein synthesis, on the generation of PSF (5). The possibility of granule basic proteins, such as cationic protein lysozyme and lactoferrin, as being precursors of PSF still remains to be elucidated.

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