ARGININE-RICH PROTEINS OF POLYMORPHONUCLEAR LEUKOCYTE LYSOSOMES

Antimicrobial Specificity and Biochemical Heterogeneity*

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Previously we reported the isolation from polymorphonuclear leukocyte (PMN) granules of a group of highly basic proteins, the electrophoretic heterogeneity of which varies from species to species (1–4). These proteins as a group have antibacterial activity against a wide variety of microorganisms, and may also contribute to the tissue-damaging properties of PMN (5–11) as well as their pyrogenicity (12) and anticoagulant activities (13, 14).

During the past 80 yr, workers have repeatedly observed that cationic proteins may possess antimicrobial effects, and it has also been found repeatedly that antimicrobial extracts can be prepared from PMN (15). Observations by Skarnes and Watson (16), by Hirsch (17), and by Cohn and Hirsch (18) are especially pertinent to the results reported in this paper. Skarnes (16) isolated antimicrobial cationic proteins from autolyzed PMN that he believed were derived from nucleohistone; this material he called leukin. Hirsch (17) recovered antimicrobial activity in citric acid extracts of PMN suspensions. He attributed this to a cytoplasmic constituent of PMN which he named phagocytin. At first, both preparations were believed to show specific antimicrobial action, related to Gram reactivity of the microbial species tested. Although the concept of antimicrobial specificity was later abandoned, Cohn and Hirsch succeeded in showing that the cytoplasmic membrane-bonded granules—the lysosome fraction—of PMN contained, in addition to acid hydrolase activity, considerable antimicrobial phagocytin activity (18).

Spitznagel and Chi (19) found that PMN lysosomes were rich in cationic protein and concluded that the antibacterial action of PMN lysosomes was associated with transfer of cationic proteins to ingested bacteria. Zeya and Spitznagel (1) showed that the antimicrobial action of PMN lysosomes is associated with their electrophoretically separable cationic proteins. They found that PMN lysosomes contain not one but several basic proteins that are different from nucleohistones and much

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more cationic than the acid hydrolases. So far no enzymatic activity has been identified with these cationic lysosomal proteins.

The electrophoretic heterogeneity of this cationic protein fraction and the multiplicity of properties attributed to it have raised several questions. We have investigated three of them: (*a*) whether the antibacterial component is a single entity with broad specificities, (*b*) whether all the cationic lysosomal components share the same antimicrobial activities, and (*c*) whether the mixture contains several components that individually exert preferential inhibitory activity against different genera and species of bacteria. Answers to these questions could provide important clues to the mode of antibacterial action of these lysosomal cationic proteins as well as to the nature of the microbiocidal deficiencies that have been described in certain diseases, for example the leukemias or the Chediak-Steinbrinck-Higashi syndrome and infectious granulomatosis of childhood (20, 21). All of these syndromes may include manifestations of PMN abnormalities and reduced resistance to infection.

In the present work, we have achieved a high degree of resolution of rabbit PMN lysosomal cationic proteins by means of sucrose density gradient electrophoresis (SDGE) and have demonstrated that the separable components differ substantially in their antibacterial specificities and in their amino acid composition. A preliminary report of this work has been presented elsewhere (22).

Material and Methods

Male New Zealand rabbits weighing 2–3 kg were used for obtaining cells. The leukocytes were recovered from sterile peritoneal exudates induced by injection of 0.5% glycogen in 0.85% saline. The lysosomal fraction was obtained by homogenization and differential centrifugation of PMN cells in 0.25 N sucrose at ice-cold temperature (18). The lysosomes were lysed in 0.01 N HCl. The lysosomal membranes and other insoluble material were removed by centrifugation at 15,000 rpm. The clear supernatant fluid containing the lysosomal proteins was dialyzed against distilled water and lyophilized.

Sucrose Density Gradient Electrophoresis.—50 mg of lysosomal protein were dissolved in 1–2 ml of buffered sucrose. The sample was then subjected to ascending electrophoresis in a sucrose density gradient column with acetate buffer, pH 4.0, ionic strength 0.01 at 700 v, 16 amp, and 20°C for 10 hr, as described elsewhere (23). The sample volume was kept small at all times to facilitate resolution of components. Fractions were collected at room temperature in an automatic fraction collector (LKB Radirac) set to deliver 5 ml per tube.

Analytic Methods.—The protein content of fractions was estimated by ultraviolet absorption at 280 mμ as well as according to the method of Lowry (24). Crystalline egg white lysozyme was used as a reference standard. The enzymatic assay for lysozyme and ribonuclease was carried out according to standard methods (23).

Paper Electrophoresis.—Electrophoresis of individual fractions was carried out in a Gelman apparatus at room temperature, 200 v and 0.02 amp on Oxoid strips (5 x 20) treated with an 0.5% aqueous solution of cetyltrimethyl ammonium bromide (CTAB) (1, 2). Acetate buffer, pH 4, ionic strength 0.05, was used. The strips were stained with Amido Black.

Antibacterial Assay.—Antibacterial assay was carried out according to a modification (22)

of the turbidimetric method of Muschel (25). The microorganisms used in the antibacterial assays have been listed in Table I. A standardized suspension of each microorganism was treated with known quantity of sample from SDGE fractions in citrate phosphate buffer, pH 5.6, for 1 hr at 37°C. After incubation, trypticase soy broth was added to the tubes and these were then returned to the water bath at 37°C. Incubation was carried out until the optical density of control tubes containing no lysosomal protein reached the range of 0.4–0.5. The per cent inhibition was measured by dividing the optical density of each tube to which some fraction had been added by the density of the control that had received no fraction, and multiplying the quotient by 100. The antibacterial activity of each fraction was then calculated in terms of a 50% inhibition end point (IEP_{50}) by plotting graphically on logarithmic probability paper the optical densities obtained in the presence of two-fold serial dilutions of the fraction. The quantity of the fraction that reduced bacterial growth by 50% in 1

TABLE I

Cultures Used to Test Antimicrobial Action of SDGE Fractions of Rabbit PMN Lysosomes

Organism	Source	Organism	Source
Proteus vulgaris	*a*	*Escherichia coli* 0117:H27	*a*
Proteus mirabilis	*a*	*Escherichia coli* O26:B6	*d*
Proteus morganii 1628	*b*	*Escherichia coli* B	*b*
Proteus morganii 1647	*b*	*Escherichia coli* K12	*b*
Staphylococcus aureus	*c*	*Streptococcus durans*	*e*
Staphylococcus aureus G.D.	*c*	*Streptococcus* Group D	*e*
Staphylococcus aureus 1-282	*c*	*Streptococcus faecalis*	*e*
Staphylococcus albus	*c*	*Streptococcus faecalis* E1	*e*

a. University of North Carolina, Department of Bacteriology Culture Collection.
b. American Type Culture Collection.
c. Kindness of Dr. G. Stewart, School of Public Health, University of North Carolina.
d. Kindness of Dr. E. Ewing, U.S.P.H.S. Laboratories, Atlanta, Ga.
e. Kindness of Dr. Harry Gooder, Department of Bacteriology.

ml of culture was taken as 1 IEP_{50} unit. The activity was expressed in terms of numbers of IEP_{50} units per mg of protein as determined by the Lowry technique.

Amino Acid Analysis.—Fractions from the leading end, middle portion, and trailing end of the cationic peak 4 (Fig. 1) were concentrated by lyophilization. Amino acid analysis was carried out according to the method of Spackman et al. (26) in an automatic Beckman Spinco, model 120 B, amino acid analyzer. For hydrolysis, 1–1.5 mg of protein were treated with 6 N HCl in evacuated sealed tubes at 110°C for 22 hr.

RESULTS

Electrophoretic Separation of Lysosomal Proteins.—Fig. 1 shows the relative protein concentrations in the fractions obtained from one electrophoretic separation. Four regions of high protein concentration were identified in the column effluent and designated as peaks 1, 2, 3, and 4 from anode to cathode. Each of the two most cationic of these groups of peaks could be further divided

into two subgroups of peaks termed 3a, 3b, 4a, and 4b. The light absorbance of all of the fractions was also determined at 2800 A and plotted in Fig. 1. The curve thus obtained differed substantially from that produced by the Lowry method because proteins in the most cationic of the major groups of fraction 4 and 3b did not absorb much light at 2800 A. As will be shown below, this is due to the lack of tyrosine and other aromatic amino acids in the group 3b and 4 fractions. This feature clearly distinguishes these very cationic proteins from known enzyme proteins, basic and otherwise, found in the complex mixture of lysosomal proteins.

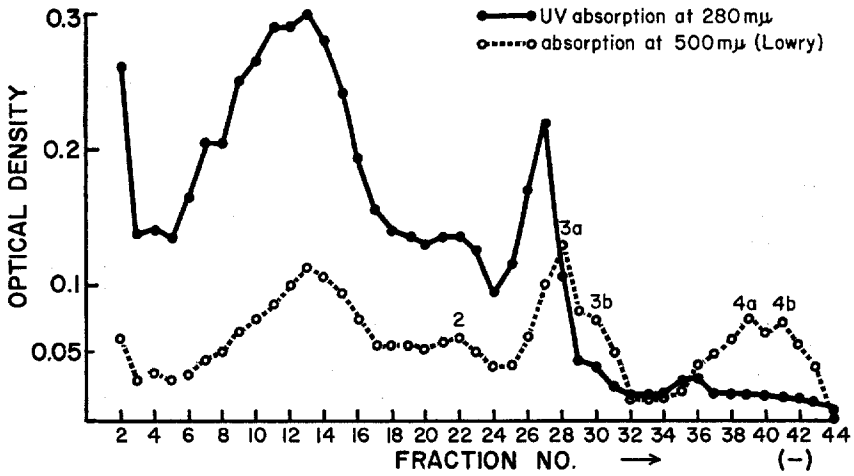

FIG. 1. Sucrose density gradient electrophoresis on acid extracts of PMN granules after 10 hr. Conditions: Sample volume 1.5–2.0 ml, acetate buffer, pH 4, ionic strength 0.01, 700 v, 20 ma, 10 hr at room temperature. The UV absorption curve (280 mμ) of fractions is compared with absorption curve of proteins at 500 mμ (Lowry). The cationic fractions between tube No. 29 and tube No. 44 show poor UV absorption at 280 mμ.

Paper Electrophoresis of Fractions Obtained by SDG Electrophoresis.—In order to assess the degree of resolution that was achieved by SDGE, the proteins in the various tubes were analyzed by cellulose acetate paper electrophoresis. Because this report is principally concerned with the antibacterial substances of PMN lysosomes only, the paper pherograms of antibacterial peaks 3b, 4a, and 4b are shown in detail (Fig. 3). Unfractionated rabbit PMN lysosomal extracts usually give about 14 distinct bands on paper electrophoresis (Fig. 3). These bands have been assigned roman numerals beginning with the most cathodal and proceeding toward the anode.

By the criterion of paper electrophoresis, SDGE achieved a high degree of resolution of the lysosomal cationic proteins and most of the fractions were

reasonably homogeneous. Only components VI and VII showed enzymatic activity. Component VII proved to be a highly purified lysozyme with specific activity almost six-fold greater than that of crystalline egg lysozyme. Component VI showed ribonuclease (RNAase) activity but recent evidence[1] indicates that what is seen as VI on the pherograms is actually another low molecular weight (4000–5000) protein with no known enzymatic function and with electrophoretic mobility similar to that of RNAase.

Distribution of Antibacterial Activities.—Four genera of bacteria, two Gram-negative (*Proteus vulgaris* and *Escherichia coli*) and two Gram-positive (*Staphylococcus aureus* and *Streptococcus faecalis* Group D) were selected for the

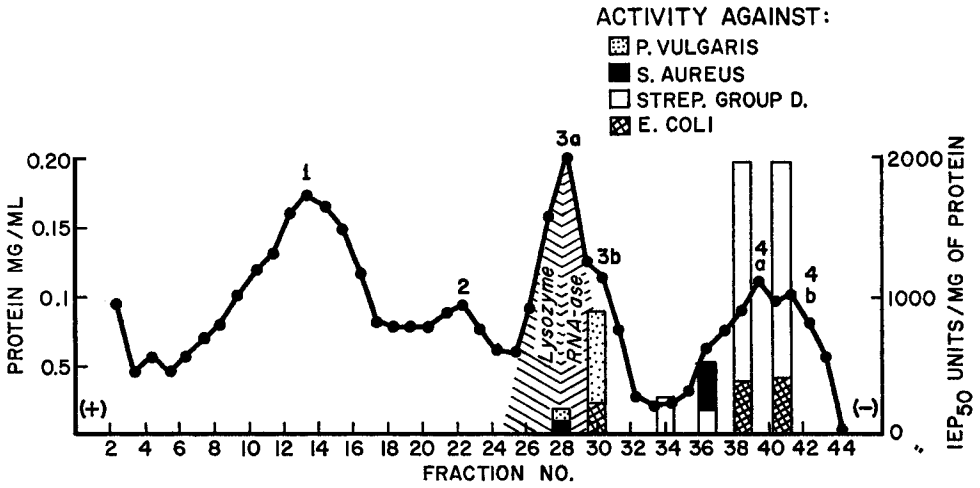

FIG. 2. Sucrose density gradient electrophoresis of acid extract of PMN granules. The antibacterial activities against four microbial genera were distributed differentially in protein peaks 3 and 4.

antibacterial assay of effluents from the column. It is important to note that, within a mixture of proteins migrating in an electric field, only the leading edge of the fastest migrating peak can be homogeneous while the slower peaks, because of trailing and overlapping effects, are contaminated to varying degrees by the more rapidly migrating components. Under these conditions it is comparatively easy to assess the smallest amount of protein in a leading fraction that will inhibit a particular microorganism. The other extreme case, the measurement of the highest noninhibitory concentration of proteins in the trailing fractions, has obvious limitations.

Analysis of the column effluent (Fig. 2) showed that the antibacterial activi-

[1] Zeya, H. I., and J. K. Spitznagel. Biological and biochemical characterization of a purified component–cationic component I of PMN lysosomes. In preparation.

ties against the four genera of microorganisms were confined to the areas under peak 4a and 4b and those under peak 3b, where the protein had little capacity to absorb light at 2800 A (Fig. 1). Lethal activities against the several microorganisms were localized in different parts of the column effluent. *S. faecalis* Group D was maximally inhibited by components in the leading edge of peaks

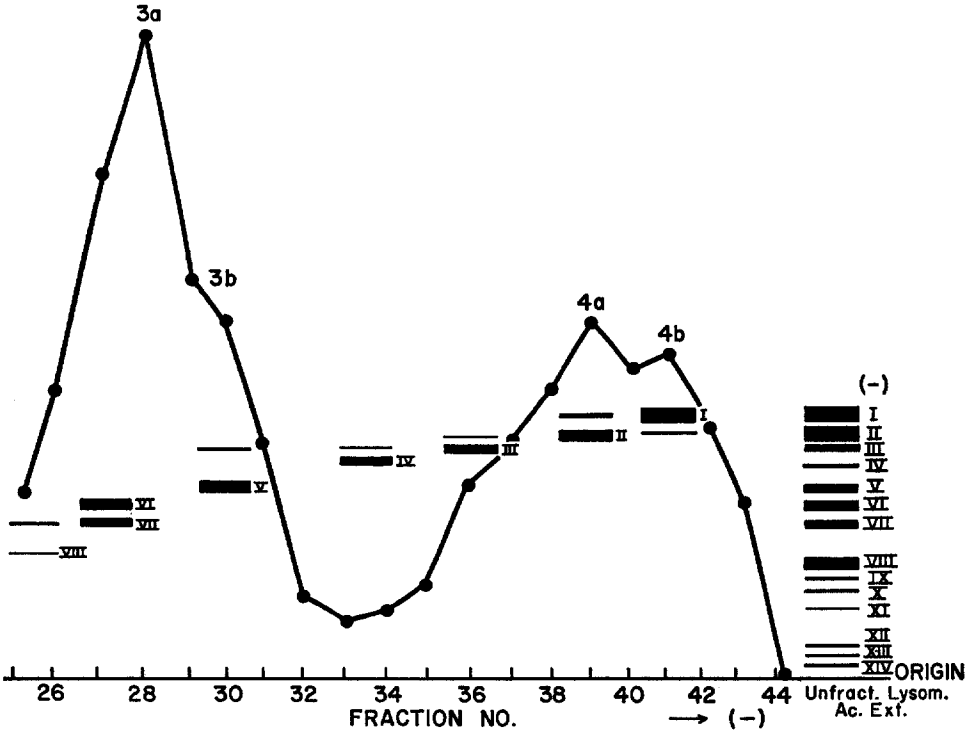

FIG. 3. Cellulose acetate electrophoresis of effluent fractions obtained from sucrose density gradient electrophoresis column. Conditions of cellulose acetate electrophoresis pH 4, ionic strength 0.05, 200 v, 0.02 amp. The sequential arrangement of electrophoretically identifiable components in effluent fractions corresponds to components I to VII of unfractionated lysosomal acid extracts (pherogram of unfractionated lysosomal extract is at far right).

4a and 4b. Peak 4b contained component I in a high state of purity (Fig. 3) whereas the fraction at peak 4a contained a high concentration of component II and a trace of component I. This indicated that components I and II, either singly or in combination, were active against *S. faecalis*. The bacteria were highly sensitive to these components (2000 IEP_{50} units/mg of protein) and even in such small quantities the components exerted a bactericidal activity against *S. faecalis*.

Against *S. aureus*, components I and II were ineffective. These proteins did not inhibit *S. aureus* even when used at concentrations 50-fold greater than the IEP_{50} for streptococci. Antibacterial activity against *S. aureus* was localized in the region of the anodal limb of peak 4 (tube No. 36) where component III made its appearance. The IEP_{50} of component III for *S. aureus* was lower than the IEP_{50}'s of components I and II for *S. faecalis*, but the degree of specificity was still marked.

Components that inhibited *P. vulgaris* were found to be at the level of peak 3b (Fig. 2). The components of peaks 4a and 4b (I, II, and III) were not inhibitory to *P. vulgaris*. The fraction that inhibited *P. vulgaris* contained component V of the lysosomal cationic proteins (Fig. 3). The anti-*P. vulgaris* activity was separated from and located in the fraction ahead of lysozyme and ribonuclease. Although we had previously reported that an additional peak of anti-*P. vulgaris* activity could be detected (22) on the anodal side of the lysozyme peak, we have not been able to confirm this finding. Further refinement of our methods of sample application, particularly maintenance of low sample volumes (1–1.5 ml) may have enhanced the resolving capacity of the SDGE.

The antibacterial activity against *E. coli* 0117-H27 was present in the region of peaks 4a and 4b, and to a smaller extent in the region of peak 3b (component V) (Figs. 2 and 3). It appeared that components I and II that inhibited *S. faecalis* also inhibited this *E. coli*. However, when they were directly compared, the components were less effective against *E. coli* than against *S. faecalis*.

Strain Differences in the Sensitivity of Microorganisms.—We next investigated whether strains or species of the genera we had tested would follow the same general sensitivity pattern to lysosomal cationic protein components. These sensitivity patterns are shown in Table II. Three species and two strains of *P. vulgaris* showed identical sensitivity patterns. Component V in the region of peak 3b was found to inhibit all proteus strains to some degree. However, there were significant differences in the susceptibility of individual organisms to component V.

Similarly the strains of *S. aureus* showed maximum sensitivity to component III with marked quantitative variations in sensitivity between the strains. One strain of *S. aureus*, 1-282, proved an exception to the general pattern of staphylococcal sensitivity. It was highly sensitive to components I and II that did not inhibit the other *S. aureus* examined. In fact strain 1-282 was sensitive to most of the cationic components, IV included. This strain was a penicillinase producer although the significance of this observation is not clear.

Interstrain differences were most evident with *E. coli*. As shown in Table II, *E. coli* 0117-H27 was maximally inhibited by components I and II (anti-streptococcal components) and to a lesser degree by component V (anti-*P. vulgaris* component), whereas *E. coli* 026-B6 was only inhibited by component V. The sensitivity pattern for *E. coli* 026-B6 was more like that of *P. vulgaris*.

E. coli B showed a pattern that seemed to be a combination of *E. coli* 0117:H27 and *E. coli* 026:B6 inhibition patterns. *E. coli* B was also sensitive to another component, IV, in addition to being susceptible to components I and II. *E. coli*

TABLE II

Differences in Sensitivity of Species and Strains of Microorganisms to PMN Lysosomal Cationic Components

Test organisms*	Cationic lysosomal protein fraction					
	I	II	III	IV	V	VI and VII (RNAase and lysozyme)
Proteus vulgaris	—‡	—	—	—	900§	200
Proteus morganii 1628	—	—	—	—	250	120
Proteus morganii 1647	—	—	—	—	450	120
Proteus mirabilis	—	—	—	—	400	120
Staph. aureus	—	—	600	—	—	120
Staph. albus	—	—	600	—	—	—
Staph. aureus G.D.	—	—	1400	—	—	—
Staph. aureus 1-282 (penicillinase producer)	1000	1000	700	2000	600	400
E. coli O117:H27	400	400	—	—	200	—
E. coli O26-B6	—	—	—	—	700	150
E. coli B	400	400	—	1500	900	300
E. coli K12	400	400	—	—	200	—
Strep. durans	2000	2000	330	500	—	—
Strep. D, F24	2000	2000	400	2000	200	150
Strep. faecalis	500	500	300	4000	800	250
Strep. faecalis E1	400	400	300	4000	500	500

* Concentration of bacteria = 10^6/ml.

‡ No inhibition at highest level tested.

§ No. of 50% inhibitory units/mg of protein. Determined by modified Muschel's turbidimetric antibacterial assay.

Fractions I and II tested at protein concentration = 100 50% inhibitory units/ml. Fractions III and IV tested at protein concentration = 300 50% inhibitory units/ml. Fraction V tested at protein concentration = 180 50% inhibitory units/ml. Fractions VI and VII tested at protein concentration = 120 50% inhibitory units/ml.

K12 was sensitive to components I and II as well as to component V, a pattern similar to *E. coli* 0117:H27.

The various streptococci also showed differences in their sensitivity to cationic components. *S. durans* was highly susceptible to components I and II and to a much lower degree to component IV, whereas two other organisms,

S. faecalis and *S. faecalis* E_1, were moderately sensitive to components I and II but exceptionally sensitive to component IV. These strains were only slightly susceptible to components III and V.

Some antibacterial activity was always discernible at the level of the lyso-

TABLE III

Amino Acid Composition of Fractions of Cationic Peak 4

Amino acids	Leading end (closest to cathode)	Middle region	Trailing end
	(Anti-streptococcal activity)		(Anti-staphylococcal activity)
	I*	II	III
	moles/100 moles		
Basic amino acids	36.6	34.4	31.8
Lysine	0.9	1.7	3.8
Arginine	31.3	30.0	25.3
Histidine	3.5	2.7	2.7
Cystine (half)	14.2	14.3	13.5
Glutamic acid	4.1	4.4	3.9
Aspartic acid	0.43	0.5	1.67
Threonine	0.8	0.4	0.77
Serine	1.4	0.8	1.7
Proline	6.7	5.8	7.0
Glycine	5.7	5.7	6.16
Alanine	8.5	8.4	9.4
Valine	4.3	4.4	4.0
Methionine	0.2	0.1	0.39
Isoleuline	7.1	6.4	6.1
Leucine	10.3	12.4	9.8
Phenylalanine	2.3	2.5	2.9
Tyrosine	0.0	0.0	0.7
Arginine-lysine ratio	34.7	17.6	6.6
Basic-acidic amino acid ratio	7.9	7.0	5.7

* Major cationic component present in the fraction (Fig. 3).

zyme and ribonuclease peaks. This could have been due either to the direct effect of the enzymes on the bacteria or, as seems more likely, could have resulted from synergism of enzymes and the contaminating cationic components.

Amino Acid Analysis in Relation to Antimicrobial Specificity.—Lysosomal components I, II, and III had shown considerable differences in antibacterial action and were available in a reasonably homogeneous state. It seemed worthwhile, therefore, to estimate their amino acid composition in the hope that some explanation might be found for their somewhat different biological properties.

The amino acid composition of the fractions taken from three different regions of cationic peak 4 is shown in Table III. Consistent with their rapid cathodal mobilities, the proteins contained high concentrations (32–36 moles/100 moles) of basic amino acids, of which arginine alone contributed 25–31 moles/100 moles. The acidic amino acid content was low and did not exceed 6 moles/100 moles. Components I, II, and III showed a lack of aromatic amino acids, especially of tyrosine. Tyrosine was virtually absent from components I and II, though a small amount of this amino acid was present in component III. Interestingly enough, a small 2800 A peak was also discernible at the level of component III (see Fig. 1). The cationic protein fractions showed an unusually high content of cystine (14%). In the fractions, the ratio of basic to acidic amino acids was high and became increasingly higher from the trailing edge (less cationic) to the leading edge (most cationic) of peak 4. There was also a marked difference in the arginine to lysine ratios of the three fractions. An unusually high ratio of 34.7 was found in the components of the leading edge of peak 4, whereas the fraction from the trailing end of the peak gave a figure of 6.6.

DISCUSSION

It is evident that the broad spectrum antibacterial activity of PMN lysosomes is not due to a single component but is distributed among a group of cationic proteins that on resolution exhibit a preferential inhibitory activity against different bacterial species. These cationic components are characterized by weak absorption of light at 2800 A owing to a lack of aromatic amino acids, especially tyrosine and probably tryptophan, and they move faster towards the cathode than lysozyme or RNAase. Components designated I, II, and III move as a group (protein peak 4) towards the cathode, and they move faster than components IV, V, and VI. Tested against various Gram-positive and Gram-negative organisms, even the components within these electrophoretically separable groups exhibit selective antimicrobial activities. Components I and II are highly effective against streptococci, 1 μg being bactericidal to 10^6 microorganisms, but do not inhibit staphylococci even with 50-fold higher concentrations of protein. Nevertheless, staphylococci are inhibited by component III of the same group. Against the proteus group, components I, II, and III are ineffective. Anti-proteus activity is associated with the slower moving components V or V and VI. On the other hand, some *E. coli* are inhibited by

components I and II, the same components that inhibit streptococci, though five times more protein is required to inhibit equal numbers of *E. coli*. The components are not exclusively antibacterial to the species of microorganisms herein described. For example, *Serratia marcescens*, another Gram-negative organism, is inhibited only by V and VI, the anti-proteus components.

Species or strains within genera usually resemble each other in sensitivity patterns. However, interstrain differences in susceptibility to lysosomal cationic proteins do exist and are especially marked with *E. coli*. One of the strains of *E. coli* was inhibited by anti-streptococcal components, another only by anti-proteus components, and yet another strain was inhibited by both. It is noteworthy that 026:B6, a smooth pathogenic strain, was only sensitive to component V. At the other extreme *E. coli* B, the roughest of the organisms that we tested and nonpathogen, was killed by all components except III.

The existence of some means of specificity in the antibacterial action of the various cationic lysosomal components suggests that they may represent a mixture of biochemically different molecular species of proteins rather than being aggregates of a single protein species. This interpretation is supported by the differences in amino acid composition of the three electrophoretically identifiable components. The amino acid composition suggests that they form a unique class of basic proteins, with a concentration of positive charges intermediate between those of histones and protamines. The lysosomal basic proteins are clearly distinguishable from nuclear histones and protamines because the former have a very high, varying arginine-lysine ratio and an unusually high concentration of cystine.

A comparison of the amino acid composition of the three components reveals considerable variation, especially in their charged groups. A varying arginine-lysine ratio (6.6 to 34.7), besides being an important indication of a true chemical heterogeneity, suggests that differences in the number and type of charged groups, and possibly in their arrangement in the protein molecule, may determine the specific biological activity of different components. This specificity may be due to the selective interaction with complementary sites on the bacterial cell wall, cell membrane, or even within the cell.

Thus the specific interaction that causes bacterial injury may be structural as well as functional. For such an interaction, the bacterial membrane may provide the most vulnerable site. The bacterial cell membrane has an anionic character that is generally due to phosphate groups of phospholipids and it is metabolically a highly active region. The bacterial respiratory activity is intimately associated with cell membrane. As suggested by Willmer (27) the structural configuration of cell membranes may vary, depending upon the relative concentration of lipid and nonlipid constituents. This might provide a suitable variety of sites for preferential interaction with the various cationic components, which might in turn affect the bacterial respiratory activity and

other functions. The lysosomal cationic proteins inhibit bacterial respiration (3) and have recently been found to be potent inhibitors of respiratory activity of rat liver mitochondria.[2] Studies are under way to determine whether some form of specificity is expressed by the cationic components in their inhibition of bacterial respiration.

In view of the large number of microbial species to which animals are exposed and the apparently limited number of cationic proteins to be found in PMN lysosomes, the specificity of individual cationic components might be expected to be rather broad. An explanation for the basis of this broad specificity may lie partly in aspects of protein structure that are not yet known. Our preliminary investigations[1] of one of the purified components have shown that it is a compound of low molecular weight (tentatively 8–10,000). This arginine-rich component contains an unusually high concentration of cystine, which suggests that the protein possesses a number of intramolecular sulfhydryl bonds and may thus be structurally complex.

The existence of specific bactericidal substances in PMN lysosomes indicates a need for investigation not only of how these substances kill different organisms but how and at what level of cell development the lysosomal cationic components are formed and whether the substances are constitutive or inducible. For example, does infection play a part in the genesis of these lysosomal cationic proteins? Studies of differences in susceptibility of various microorganisms to individual lysosomal components may shed light on the physicochemical basis of microbial pathogenicity and also interspecific differences in susceptibility to infection.

The findings reported in this paper may help to explain a newly described genetically determined disease of children (20, 21) that is characterized by frequent infection, chronic suppurative and granulomatous lymphadenitis, hepatosplenomegaly, parenchymatous infiltration especially of lungs, and episodes of infected eczematoid dermatitis. Patients with this disease have the normal spectrum of γ-globulins and normal ability to form circulating antibodies in response to antigenic stimulation. What they appear to lack is the ability of their PMN leukocytes to kill a relatively small group of microorganisms, the staphylococci that are invariably present in the suppurative lesions. Kaplan et al. (28) have recently described individual patients characterized by the selective failure of their polymorphonuclear leukocytes to kill phagocytosed staphylococci. Although there may be species differences, this is especially interesting in view of our isolation of a single anti-staphylococcal factor from rabbit PMN. Human PMN may also turn out to possess only one anti-staphylococcal protein but two or more lysosomal components that are effective

[2] Zeya, H. I., R. Pennial, and J. K. Spitznagel. Effect of lysosomal cationic proteins on mitochondrial respiration. In preparation.

against each of the other major pathogenic species. It is well known that loss of one inherited character is more common than simultaneous loss of two affecting the same over-all process. Thus, reduced resistance to staphylococcal infection after loss of a single anti-staphylococcal protein might occur more often than reduced resistance to infection by microbial species that are sensitive to two or more lysosomal proteins.

SUMMARY

The cationic antibacterial proteins of rabbit PMN lysosomes have been resolved into at least five subfractions. Each of these showed substantial selectivity in its antibacterial action against several pathogenic bacteria, including two smooth and two rough *Escherichia coli* strains, three *Staphylococcus aureus* strains, one *S. albus*, three proteus species and four different cultures of streptococcus.

Each of the subfractions possesses a different electrophoretic mobility. Amino acid analyses of the three most cationic components revealed high contents of arginine consistent with their relative electrophoretic mobilities and very high arginine to lysine ratios. Aromatic amino acids were present in very low concentrations in these proteins and their light absorption at 2800 A was correspondingly weak.

The evidence of antibacterial specificity, along with marked differences in the arginine-lysine ratios, shows that the cationic antibacterial components of rabbit PMN lysosomes are biologically and chemically heterogeneous.

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