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β-Lysin

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A brief review of the history and some of the characteristics of β-lysin will be presented before the possible role of β-lysin in the inflammatory reaction is discussed. Historically, it was shown in the latter part of the nineteenth century that serum contained thermostable substances which were bactericidal for gram-positive bacteria. In 1887, Josef Fodor demonstrated that defibrinated blood exhibited anthracidal activity. The following year, von Behring reported that the anthracidal substance in serum resisted inactivation at 60 C. In 1904, Pirenne found that the thermostable substance in rat serum which was lethal for gram-positive bacteria did not kill gram-negative bacteria. In a series of papers between 1924 and 1936, Pettersson worked on the antimicrobial spectra of sera from a variety of mammalian species. He called these thermostable substances in serum β-lysins to distinguish them from heatlabile α-lysin, alexin, or complement. In the early 1950s, working as a graduate student under the direction of Stanley Marcus, I demonstrated the total body X irradiation suppressed bactericidal activity without changing serum levels of either complement or antibody (3, 11).

The initial observation that led to the purification of β-lysin in our laboratory (1) is a typical example of serendipity. A sample of rabbit serum suspected of contamination was sterilized by Seitz filtration to illuminate the contaminating bacteria so they would not be counted in the serum bactericidal test. This Seitz-filtered serum was devoid of bactericidal activity for *Bacillus subtilis*. The filter pad with its absorbed β-lysin can be washed with large volumes of water or physiological saline solution without removing the β-lysin. The β-lysin can then be eluted from the Seitz filter pad with 1.5 M NaCl, resulting in more than a 100-fold purification of the β-

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lysin. The salt concentration of the eluate needs to be lowered by either dilution or dialysis before it is tested, since concentrations of 0.45 M NaCl or greater inhibit bactericidal activity of β-lysin. These Seitz-filter eluates of β-lysin can be further purified to as great as 37,000 times that of serum by carboxymethyl-cellulose chromatography (10).

This Seitz-filter technique has been used in our laboratories to purify β-lysins against organisms belonging to the genera *Bacillus, Clostridium, Staphylococcus, Micrococcus, Lactobacillus,* and *Arthrobacter.* Schultz (17) used this technique to isolate β-lysin against *Listeria.*

Table 1 compares β-lysin with the other two most extensively studied bactericidal systems found in serum. With the exception of members of the genus *Streptococcus,* which are resistant, most gram-positive bacteria are susceptible to the lethal action of β-lysin. It has been our experience that gram-positive organisms are never killed by the antibody-complement system, and we have yet to test a strain of gram-negative organisms which is susceptible to purified β-lysin.

In addition to gram-negative bacteria, all molds, yeasts, viruses, mammalian cells in tissue culture, and mycoplasmas were resistant to the lethal action of β-lysin.

β-Lysin differs from the two other bactericidal systems in that the serum levels of β-lysin do not equal the plasma concentrations. For example, the serum β-lysin activity against *B. subtilis* is approximately 16 times that of plasma. This increase in β-lysin against the bacilli can be explained by the fact that β-lysin is released from platelets during blood coagulation (2, 4, 8, 19). Even though serum

TABLE 1. *Comparison of serum bactericidal systems*

Parameter	Antibody-complement	Lysozyme	β-Lysin
Bactericidal spectrum	Gram-negative	Gram-positive	Gram-positive
Serum concentration (rabbit)	Variable, = plasma	1 to 2 μg/ml, = plasma	32 units/ml, 16 × plasma
Origin	Ab-plasma cells	Lysosomes	Platelets, ?
Chemical nature	Proteins and glyco-proteins	Basic protein	Basic protein
Molecular weight	—	15,000	6,000
Site of action	—	Cell wall	Cell membrane
Mode of action	Cascading enzymatic reactions	Enzymatic reaction	Nonenzymatic (similar to histones)

is more bactericidal than plasma for both *Staphylococcus* (10) and *Listeria* (17), attempts to prove a platelet origin for the β-lysins against these two types of organisms have been unsuccessful.

β-Lysin is a highly reactive cationic protein with a molecular weight of approximately 6,000. In normal serum, it appears to be attached to the serum proteins since it is distributed in all protein fractions after either ammonium sulfate fractionation or Sephadex gel filtration of serum (6). When purified β-lysin is placed on a Sephadex column in the absence of other proteins, it attaches to the beads. It will not pass through the column unless the ionic strength of eluting fluid is about 10 times the physiological concentration. Consequently, a 1.5 M NaCl solution was used to estimate the molecular weight of β-lysin (10).

The cell membrane is considered to be a primary site of action of β-lysin since: (i) β-lysin combines with purified cell membranes (7); (ii) β-lysin treatment prevents the development of the space between the wall and the plasma membrane which normally occurs in cells suspended in hypertonic sucrose solutions (12); (iii) protoplasts are readily ruptured by β-lysin (13); (iv) freeze-etch preparations of β-lysin-treated cells have wrinkled and pitted plasma membranes (we have yet to see a normal membrane on a β-lysin-treated cell or a pitted membrane on an untreated cell); and (v) there is a complete loss of unit structure when purified cell membranes are treated with β-lysin and examined by use of thin sections and electron microscopy (7).

The exact mode of action of β-lysin is unknown, but appears to be of a nonenzymatic nature. Temperature and pH curves which are characteristic of enzyme reactions cannot be obtained with β-lysin. β-Lysin may be similar to other cationic bactericidal agents such as protamine sulfate, histones, and cetyltrimethylammonium bromide. These agents are similar in that all of them act on purified cell membranes by stimulating or exposing the membrane adenosine triphosphatase of *B. subtilis* (16). β-Lysin resembles the histones in both charge and molecular weight.

The release of β-lysin in the inflammatory response may be of particular significance to the host defenses because of the low levels of β-lysin found in normal body fluids (9). Myrvik's group reported (14, 15) that sera from humans during acute-phase reactions had elevated lethal activity for *B. subtilis*.

Table 2 compares the levels of plasma β-lysin with the free β-lysin found in the peritoneal exudates of rabbits at different times

TABLE 2. *Extracellular β-lysin in peritoneal exudates induced by glycogen*

Prepn	N (mg/ml)	Units/ml	Specific activity[a]	Relative activity[b]
Plasma	9.4	1	0.11	—
Exudate				
0 time	—	<1	—	—
6 h	0.55	2	3.6	33
12 h	0.91	8	8.8	80
18 h	1.54	16	10.4	95
24 h	2.31	32	13.9	126

[a]Specific activity = units of β-lysin/milligrams of N.
[b]Relative activity = specific activity of exudate/specific activity of plasma.

after the intraperitoneal injection of 500 ml of 0.1% glycogen saline. Free β-lysin cannot be detected in the peritoneal fluid prior to an inflammatory stimulation, as shown in the second line of Table 2. The β-lysin concentration in the exudate increased to 32 units/ml by 24 h after the glycogen saline injection. This is 32 times the plasma β-lysin concentration per unit volume. The increase in specific activity which is a reflection of the bactericidal activity per milligram of nitrogen, is even more pronounced. In fact, the specific activity of the 24-h exudate is 126 times greater than the specific activity of the plasma. It is obvious from these results that the increase in β-lysin in the peritoneal exudate cannot be due to a simple diffusion phenomenon.

The increases in free β-lysin found in inflammatory exudates induced by either single or multiple subcutaneous injections of 0.1% aluminum silicate are recorded in Table 3. One of the major obstacles in obtaining these measurements is the difficulty encountered in collecting the inflammatory exudate. Even if as much as 100 ml of inducing fluid is injected subcutaneously into a rabbit, it is still necessary to inject 10 ml of saline solution into the inflammatory site immediately before collection in order to aspirate 3 to 5 ml of fluid. For this reason, these exudates might best be described as

TABLE 3. *Extracellular β-lysin in inflammatory exudates induced by subcutaneous injections of aluminum silicate*

Prepn	N (mg/ml)	β-Lysin (units/ml)	Specific activity[a]	Relative activity[b]
Plasma	9.4	1	0.11	—
Wash, 0 time	—	<1	—	—
Exudate, 18 h				
Single injection	0.57	2	3.5	32
Six daily injections	0.63	8	13	118

[a]Specific activity = units of β-lysin/milligrams of N.
[b]Relative activity = specific activity of exudate/specific activity of plasma.

subcutaneous washes. Such an exudate or wash from nonstimulated subcutaneous tissues had less than 1 unit of β-lysin per ml. By 18 h after a single injection of aluminum silicate, the β-lysin concentration had increased to 2 units of activity, which was twice the activity of plasma. If the aluminum silicate injections were repeated every 24 h for 6 days, the β-lysin level in the exudate wash was eight times that of plasma. As in the case of the peritoneal exudate, the β-lysin activity per unit of nitrogen or specific activity of the exudate increased even more than the activity per unit volume. The β-lysin specific activity of the exudate reached 32 times that of plasma after a single injection and 118 times that of plasma after multiple injections.

A number of different phenomena may account for the increase in β-lysin at inflammatory sites. The coagulation that results in the thrombi formation in capillaries and in lymphatics draining inflammatory sites may cause a local release of β-lysin. Another factor that might indirectly increase the β-lysin concentration of an inflammatory exudate is any antigen-antibody reaction that takes place in inflammatory sites. The intravenous injection of a bovine serum albumin (BSA)-anti-BSA complex into a normal rabbit or of BSA into a BSA-immunized rabbit caused 32-fold increases in the free plasma β-lysin (18).

The presence of bacteria in an inflammatory site might also result in the release of β-lysin. Bacteria injected intravenously or added to whole citrated blood caused eight- and four-fold increases in the plasma β-lysin. The release of β-lysin after the injection of bacteria or the in vivo antigen-antibody reactions was accompanied by morphological damage to platelets and a decrease in the number of circulating platelets. Phagocytosis may play a role in the release of β-lysin induced by bacteria or antigen-antibody reactions since the β-lysin release was always accompanied by an increase in lysozyme. It is assumed that this lysozyme was released along with other lysosomal enzymes during the degranulation that accompanies phagocytosis. Another observation which gave indirect support to phagocytosis playing a part in β-lysin release was that bacteria added to citrated platelet-rich plasma did not cause the release of β-lysin which followed the addition of bacteria to citrated whole blood.

Table 4 contains results collected by Robert R. Roberts in our laboratory on what happens when citrated cell-free plasma or platelet-rich plasma is reacted with 1,000 μg/ml concentrations of 14 different enzymes for 30 min at 37 C. The β-lysin concentration of the

TABLE 4. *Effect of enzymes on release of β-lysin from platelets*

Treatment[a]	β-lysin concn (units/ml) after enzyme treatment	
	Cell-free plasma	Platelet-rich plasma
Controls (no enzyme)	2	4
Streptokinase	2	64
Neuraminidase	2	32
Papain[b]	2	32
Phospholipase C	2	32
Sulfatase[b]	1	16
Elastase	2	8
Phosphatase	2	8
Protease[b]	2	8
Ribonuclease	2	8
Carboxypeptidase B	—	4
Hyaluronidase	2	4
Lipase	2	4
Pepsin	2	4
Fibrinolysin[b]	1	1

[a]Treatment was for 30 min at 37 C.
[b]These enzymes inactivate purified β-lysin.

cell-free plasma was the same as that of the platelet-rich plasma before the incubation. As can be seen in the first line of Table 4, some β-lysin is spontaneously released from the platelets during the 30-min incubation. The β-lysin of cell-free plasma was never increased by enzyme treatment, but was lower in two of the four enzymes which inactivate purified β-lysin. In contrast, 9 of the 14 enzymes caused platelets to release β-lysin at an accelerated rate. Streptokinase, the most active of these enzymes, caused platelets to release 16 times as much β-lysin as was found in platelet-rich plasma which was incubated in the absence of enzyme. Neurominidase, papain, and phospholipase C caused an eight-fold increase in the release of β-lysin. Sulfatase caused a fourfold increase, and elastase, phosphatase, protease, and ribonuclease A doubled β-lysin release. The role of any of these enzymes in the in vivo release of β-lysin is strictly speculative at this time. The results do open the possibility that β-lysin may be released in an inflammatory site from platelets as a result of the enzymes in inflammatory exudates. These enzymes could be of host or microbial origin.

Even though β-lysin has never been found to be lethal for gram-negative organisms by itself, recent findings (5) in our laboratory have shown that β-lysin works in conjunction with both lysozyme and the antibody gram system in killing gram-negative bacteria. Consequently, the role of β-lysin in the inflammatory reaction is not limited to its effect on gram-positive bacteria.

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