PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 153, 49-51 (1976)

The Mechanism of Inhibition of Aminoglycoside and Polymyxin Class Antibiotics by Polyanionic Detergents (39478)

STEPHEN C. EDBERG, CECIL J. BOTTENBLEY, AND JACQUES M. SINGER

Division of Microbiology, Department of Pathology, Montefiore Hospital and Medical Center, 111 East 210th Street, New York, New York 10467

Polyanionic detergents have been incorporated into blood culture media for many years (1). Sodium polyanetholsulfonate (SPS), also known as Liquoid, has been the most extensively used and studied member of this class of compounds; it has been shown to possess antiphagocytic (2), anticomplementary (3), antiprotein (4), and antiantibiotic activity (5, 6). SPS exhibits little antibacterial activity, with the family *Enterobacteriaceae* and genus *Staphylococcus* unaffected by concentrations as high as 1.25% (7). Only the anaerobic streptococcus *Peptostreptococcus anaerobius* (8) and occasional strains of *Neisseria meningitidis* (9) are inhibited by SPS. Sodium amylosulfonate (SAS), although recently introduced and not extensively studied, appears to have the same activity spectrum as SPS (10).

SPS appears to effectively obviate the activity of cationic compounds. Positively charged antibiotics, such as aminoglycosides and polymyxins, and positively charged proteins, such as lysozyme and beta-lipoprotein, are selectively affected (10). The mechanism of action by which polyanionic detergents inhibit antibiotics has not been studied directly. Studies in the literature have utilized an indirect indicator system, the modification of bacterial growth, to measure the effect of detergent on antibiotic activity. As a result, the mode of inhibition of antibiotics by polyanionic detergents remains undetermined. Accordingly, a study was undertaken to determine the mechanism of action; in lieu of an intermediate indicator system a non-antigen–antibody double diffusion system in agar gel, originally described by Kunin and Tupasi (11), was employed. Since the reaction milieu can exert an effect on the activity of charged materials all experiments were conducted in inhibitor-free media.

Methods. Diffusion plates were made by pouring 5 ml of 1.5% agarose in deionized distilled water into 60 × 15 mm plastic tissue culture dishes. Seven wells were cut using a template (Grafar Company, Detroit, Michigan); the six outer wells were 3 mm in diameter, the single inner well 4 mm in diameter. Inner wells contained a detergent concentration; the six outer wells contained a geometric dilution series of the tested antibiotics. All plates were used within 48 hr of pouring. Antibiotic and detergent solutions were added to the wells and the plates were incubated at both 4 and 22°C for 48 hr.

Antibiotics (Canalco, Rockville, Maryland), obtained as standard powders without preservatives, included the aminoglycosides streptomycin, kanamycin, gentamicin, and tobramycin, the polymyxins colistin and polymyxin B, and penicillin G. Antibiotic concentrations of 10,000, 5,000, 2,500, 1,250, 625, and 312 μg/ml were tested. Sodium polyanethol sulfonate (Hoffmann-LaRoche, Nutley, New Jersey), sodium lauryl sulfate (SLS) (Mann Research Labs., New York, New York), and disodium 4-dodecylated oxydibenzenesulfonate (Benax 2A1, Dow Chemical Company, Midland Michigan) represented the polyanionic group. The nonionic and cationic detergents tested were tween 80 (Atlas Chemical Ind., Wilmington, Delaware) and benzalkonium chloride (Winthrop Labs., New York, New York) respectively. Detergent concentrations included 50,000, 12,500, 10,000, 6,250, 3,125, 1,560, and 780 μg/ml. All antibiotics and detergents were diluted in deionized distilled water and used within 2 hr of constitution.

All plates were observed at 1, 4, 18, 24, and 48 hr incubation. Lines of precipitation were recorded manually and photographically.

In order to establish that precipitation between antibiotic and polyanionic detergent

effectively reduced the amount of active antibiotic present in the milieu, a tube dilution system was constructed. Polyanionic detergents were geometrically diluted from 1 to 0.03% in distilled water. To each test tube containing a polyanionic detergent an equal volume of 0.5% antibiotic was added. After 4 hr of incubation at room temperature, the tubes were centrifuged. The supernatant was tested for free antibiotic and the pellet, after washing five times in distilled water, was resuspended and tested for bound antibiotic (12).

Results. All polyanionic detergents formed precipitin lines with streptomycin, kanamycin, gentamicin, tobramycin colistin, and polymyxin B (Table I). There were quantitative differences in both the lowest concentration of polyanionic detergents yielding a precipitin line and in the strengths of the precipitin lines. Neither SLS, SPS, nor Benax produced precipitin lines with penicillin. Figure 1 demonstrates a typical non-antigen-antibody precipitin line formed between a polyanionic detergent, SPS, and an aminoglycoside antibiotic, kanamycin. The precipitin line is analogous to that seen with Ouchterlony double diffusion between antigens and antibodies. All reactions between polyanionic detergents and aminoglycosides and polymyxins yielded only one precipitin band. In all cases precipitation resulted in the inactivation of antibiotic.

Most reactant concentrations produced visible lines of precipitation within 4 hr both at 22 and 4°C. Precipitin lines increased in density with time, reaching a maximum at 24 to 48 hr. All three polyanionic detergents were able to detect the lowest concentration antibiotic although the reaction was strongest with SLS. It thus appears possible that the detection of antibiotics may be possible by varying well diameters, the juxtaposition of the wells, and the buffer system. Benax was the least sensitive of the polyanionic detergents being able to detect 312 μg/ml of aminoglycoside at a concentration of 10,000 μg/ml.

As would be expected, reactions were both stronger and more sensitive with the larger, higher molecular weight polypeptide, antibiotics colistin and polymyxin B. Here again SLS was the most active followed by SPS, while Benax was considerably less active (Table I).

Neither the nonionic detergent, tween 80, nor the cationic detergent, benzalkonium chloride, yielded visible precipitin lines with any of the antibiotics tested (Table I).

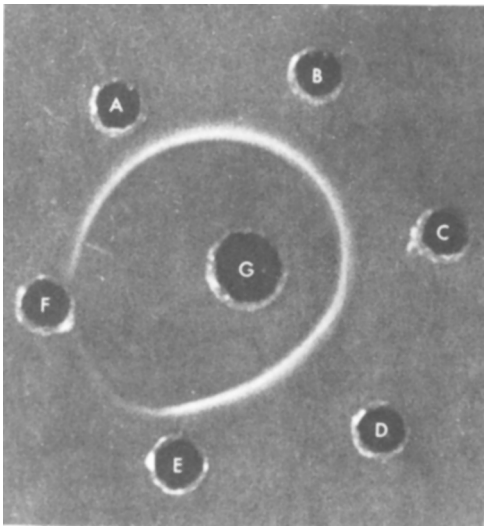

FIG. 1. Precipitation band formed between SPS and Kanamycin. Kanamycin concentrations are in the outer wells (A = 10,000 μg/ml, B = 5,000 μg/ml, C = 2,500 μg/ml, D = 1,250 μg/ml, E = 625 μg/ml, and F = 312 μg/ml) with 10,000 μg/ml of SPS in the inner well (G).

TABLE I. INTENSITY OF THE PRECIPITATION REACTION BETWEEN DETERGENTS AND ANTIBIOTICS.[a]

	STR[b]	KM	GM	TB	COL	POL	PEN
SPS	2+	2+	2+	2+	3+	3+	0
SLS	3+	3+	3+	3+	4+	4+	0
Benax 2A1	1+	1+	1+	1+	2+	2+	0
Tween 80	0	0	0	0	0	0	0
Benzalkonium chloride	0	0	0	0	0	0	0

[a] 4+ represents the strongest reaction observed in this series (2500 μg/ml POL and 2500 mcg/ml SLS).
[b] Antibiotic abbreviations: STR—streptomycin; KM—kanamycin; GM—gentamicin; TB—tobramycin; COL—colistin; POL—polymyxin B; PEN—penicillin G.

Discussion. It has been established that polyanionic surfactants are able to inhibit the normal functioning of several biological systems and the activity of selected classes of antibiotics. Numerous studies using indirect biological markers have demonstrated the inhibitory effect of SPS on polymorphonuclear leukocytes (2, 13) and complement (13). Investigations concerning the reaction mechanism between polyanionic detergents and antibiotics have not been published. Following procedures originally presented by Kunin and Tupasi (11) it was found that polyanionic detergents inhibit aminoglycoside and polymyxin classes of antibiotics by combining directly with them to form a precipitate. All three polyanionic detergents tested, although demonstrating quantitative differences in their inactivating abilities, gave precipitation lines with all members of both antibiotic classes.

Neither the nonionic detergent, tween 80, nor the cationic detergent, benzalkonium chloride, was found to react with any of the antibiotics tested. It would appear that precipitation, and concomitantly inactivation, is the direct result of the interaction of a negatively charged detergent with a positively charged antibiotic, to form an insoluble polymer. Apparently at least two positively charged amino groups on the antibiotic molecule are available for bonding with the negatively charged groups on the detergent to create a lattice-like structure which then forms a precipitate. Unlike other methods utilized to inactivate aminoglycoside antibiotics, such as the addition of calcium to bacteriological media, which indirectly affects the antibiotic by changing bacterial cell wall permeability (15), polyanionic detergents inactivate aminoglycosides and polymyxins directly, with no effect on bacteria.

It appears theoretically possible (16) to develop a quantitative antibiotic assay based on the formation of non-antigen–antibody precipitation lines since only one band of precipitation is formed and the density and position of the band vary in direct relationship with the concentration of the reactants.

Summary. Polyanionic detergents, the most widely used of which is sodium polyanethol sulfonate (SPS), inhibit polymorphonuclear leukocyte, complement, lysozyme, and antibiotic activity. SPS has been utilized for years in the culture of blood for bacterial pathogens. Utilizing an agarose gel double diffusion system it was ascertained that polyanionic detergents, as represented by SPS, sodium lauryl sulfate, and disodium 4-dodecylated oxydibenzene-sulfonate, inactivate antibiotics by combining directly with them to form a precipitate. Only the positively charged aminoglycoside and polymyxin classes of antibiotics were affected. Neither nonionic nor cationic detergents interacted with aminoglycoside antibiotics. It would appear that a polymer is formed with both the polyanionic detergent and antibiotic each at least divalent. The reaction is independant of and does not interfere with bacterial growth.

1. Auxilia, F., Riv. Clin. Peidat. **32,** 818 (1934).
2. Fleming, A., Lancet **2,** 434 (1943).
3. Lowrance, B. L., and Traub, W. H., Appl. Microbiol. **17,** 839 (1969).
4. Bergamini, L., and Ferrari, W., Boll. Inst. Sieroter. Milan. **27,** 89 (1948).
5. Traub, W. H., Experientia **25,** 206 (1969).
6. Traub, W. H., and Lowrance, B. L., Experientia **25,** 1184 (1969).
7. Yourassowsky, E., Compt. Rend. Soc. Biol. **158,** 215 (1964).
8. Hoare, E. D., J. Pathol. Bacteriol. **48,** 573 (1939).
9. Eng, J., and Iveland, H., J. Clin. Microbiol. **1,** 444 (1975).
10. Kocka, F. E., Margoc, T., and Searcy, R. L., Ann. Clin. Lab. Sci. **2,** 470 (1972).
11. Kunin, C. M., and Tupasi, T., Proc. Soc. Exp. Biol. Med. **133,** 858 (1970).
12. Edberg, S. C., and Chu, A., Amer. J. Med. Tech. **41,** 99 (1975).
13. Belding, M. E., and Klebanoff, S. J., Appl. Microbiol. **24,** 691 (1972).
14. Traub, W. H., and Lowrance, B. L., Appl. Microbiol. **20,** 465 (1970).
15. Ervin, F. R., and Bullock, W. E., Ant. Ag. Chemo. **61,** 831 (1974).
16. van Oss, C. J., and Fontaine, M., Z. Immun. Exp. Ther. **122,** 44 (1961).

Received February 5, 1976. P.S.E.B.M. 1976, Vol. 153.