Interaction of Quaternary Ammonium Bactericides with Biological Materials I: Conductometric Studies with Cephalin

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Abstract □ Quaternary ammonium surfactants are more active against Gram-positive organisms than against Gram-negative bacteria. This difference has been attributed to the presence of cephalin in the cell wall of the Gram-negative bacteria. This paper presents a review of previous *in vivo* work reported in the literature. Additionally, as a preliminary step in elucidating the mechanism behind this difference by studying the molecular interactions between these bactericides and bacterial wall constituents by use of an *in vitro* film balance approach, a conductometric study on cephalin and certain alkylbenzyldimethylammonium chlorides was undertaken. An interaction in solution was observed, the data indicating that two molecules of cephalin react with one molecule of the quaternary ammonium surfactant.

Keyphrases □ Quaternary ammonium bactericides—interaction with cephalin, *in vivo* literature review, *in vitro* conductometric study □ Cephalin interaction with quaternary ammonium bactericides—*in vivo* literature review, *in vitro* conductometric study □ Conductometric studies—interaction of cephalin with quaternary ammonium bactericides □ Bactericidal activity—quaternary ammonium compounds

Since the original observation by Domagk (1) that the alkylbenzyldimethylammonium chlorides were extremely potent antibacterial agents, much work has been devoted to the action of cationic surface-active quaternary ammonium bactericides. Numerous structural modifications of the original series were carried out in an effort to increase the bactericidal activity of the class. In addition, many studies were conducted to determine the manner in which these compounds exert their action. However, the original material, alkylbenzyldimethylammonium chloride, also known collectively as benzalkonium chloride, is still one of the most widely used agents.

LITERATURE REVIEW

It was observed (2, 3) that the quaternary ammonium compounds are active against Gram-positive and Gram-negative organisms as well as fungi. However, the Gram-positive organisms show a higher degree of susceptibility to these agents than do the Gram-negative organisms (2, 3). The resistance of the Gram-negative organism has been ascribed to the phospholipid present in its cell envelope (4), which appears to be of the highly acidic cephalin type. The cell wall of the Gram-positive organism lacks such a phospholipid component.

Several observations tend to strengthen this theory. It was observed (5) that prior addition of phospholipids to a media containing Gram-positive cells can prevent the inhibition of metabolism by cationic surfactants. Furthermore, exposure of the cells to cephalin, followed by a washing of the cells, also protected the organisms. Based on these observations, it was postulated that the phospholipid was adsorbed onto the bacterial surface, where it subsequently interacted with the bactericide, rendering it inactive. It also was shown (6) that the prior addition of protamine to a media of Gram-negative organisms markedly increased the susceptibility of these organisms to quaternary ammonium bactericides. The highly basic protamine was shown to form an insoluble precipitate with cephalin (7). Such experimental evidence suggests that the phospholipid portion of the complex cell wall is responsible for the greater degree of protection afforded the Gram-negative bacteria.

Salton (8) proposed that the action of lytic agents on bacteria is as follows: (*a*) adsorption and penetration of the porous wall, (*b*) interaction with lipid–protein membrane, (*c*) leakage of low molecular weight metabolites, (*d*) degradation of proteins and nucleic acids, and (*e*) lysis due to wall-degrading autolytic enzymes. Other studies (4, 9, 10) showed that the cell membrane, rather than the cell wall, is the primary site for the bactericidal action of lytic agents. However, since the cell membranes for the Gram-positive and Gram-negative organisms are in both cases lipoprotein, it would appear that the difference in susceptibility should be ascribed to the difference between the cell walls. Hence, the interaction with the cell wall is of primary importance to the bactericidal action of the compound.

It has been established that the antibacterial action of the quaternary ammonium salts is related to their physical, rather than chemical, properties. The most significant physical property of these compounds is their surface activity. Numerous authors studied the interfacial properties of the antibacterial quaternary ammonium bactericides and attempted to relate these properties to the *in vivo* activity. Zissman (11) compared the minimum effective concentration (MEC) of a series of quaternary ammonium bactericides with surface tension lowering and found that solutions having the same MEC had surface tension values of the same order of magnitude. Others (12, 13) attempted to relate CMC data to the thermodynamic activity of the solution and, in turn, to relate this derived thermodynamic activity to the MEC. As an extension of this work, the Ferguson principle (14) was used to explain the dependence of the MEC on the surface activity.

Ferguson (14) proposed that the chemical potential of a series of substances possessing "physical toxicity" could be used as a toxic index. His theory was based on the fact that at equilibrium the chemical potential and, hence, activity of the substance in the internal and external phases must be equal. In the case of quaternary ammonium bactericides, the external phase is the bulk solution and the internal phase is the cell membrane of the bacteria. Ferguson also showed that the activity at the toxic concentration could be approximated by S_t/S_o, where S_t is the molar concentration of the toxic substance and S_o is its solubility. Thus, if the Ferguson principle holds true, it substantiates the theory that the bactericidal activity is the result of some physical activity rather than its chemical reaction.

Other workers similarly attempted to invoke the Ferguson principle to explain bactericidal activity. Ecanow and Siegel (12) attempted to define the thermodynamic activity as the ratio of the MEC to the CMC. For three quaternary ammonium bactericides of equal alkyl chain length but different polar head groups, Weiner and Zografi (15) defined the activities as the ratio of the Gibbs surface excess at the MEC to that at the CMC. Good agreement was found between the activities of all three quaternary ammonium bactericides when tested against Gram-positive and Gram-negative organisms and fungi. These authors concluded that the mechanism of action of these compounds depends primarily on a physical relationship between the external phase and the internal phase.

In an effort to understand the nature of the interactions that occur between drugs and biological tissues, various authors proposed the use of systems; probably the most useful of these systems is an insoluble monolayer adsorbed at the air/water interface. The first use of insoluble monolayers as models for biological membranes appears to be that of Schulman and Rideal (16). These authors used spread monolayers of gliadin and gliadin–cholesterol as a model of the cell wall of a human red blood corpuscle. They then studied the interaction of hemolytic and agglutinating agents in the subphase on the spread monolayers. The penetration into,

as well as association with, an insoluble monolayer by these compounds was monitored as a function of the increase in surface pressure or area and change of surface potential with time. Using criteria established in these studies, a relationship was established (17) between a compound's effect on the erythrocytes and the nature of its interactions with an insoluble monolayer.

The use of monolayers to simulate biological membranes has become greatly expanded since the original work of Schulman and Rideal (16). Since the original investigations into the mechanism of hemolysis, several authors have attempted to correlate monolayer penetration with the activity of other classes of drugs including the sulfas, local anesthetics, tranquilizers, and antibiotics.

Although a great deal is known about the end results of the action of quaternary ammonium compounds on bacteria, *i.e.*, cellular leakage and lysis, and postulates have been presented on the manner in which bacterial death is caused, little is known about the nature of the interactions between the bactericide and the cellular substituents. However, it does appear, as stated by Schulman *et al.* (18), that the greater resistance of Gram-negative over Gram-positive organisms is due to the higher lipid content in the bacterial cell walls of the Gram-negative cell. Many studies using live bacteria have been carried out in an effort to gain insight into the mechanism of action of the cationic bactericides. Under such conditions, it is difficult to determine the nature of the interactions between the bactericide and the cell wall and membrane components that ultimately lead to bacterial cell death.

EXPERIMENTAL

Since the use of insoluble monolayers to simulate cell structures and subsequent study of the action of drugs on these monolayers have been well established in the literature, the purpose of this investigation was to study the interaction that occurs between a quaternary ammonium bactericide and the components of a simulated bacterial cell wall. These studies were carried out through the use of insoluble monomolecular layers and a conductometric analysis of solutions of biological materials.

The model membrane chosen to simulate the Gram-positive cell wall was that of an insoluble monomolecular film of the insoluble protein gliadin spread at the air/water interface. A protein–phospholipid monolayer of gliadin and cephalin was used to simulate the Gram-negative bacterial wall. By studying the interactions that occur between these spread monolayers and the surface active bactericide injected beneath, the role of the components in determining the susceptibility of the organism may be assessed.

In addition, a conductometric study was carried out to establish the presence of an interaction between the bactericide and the cephalin. For this study, both materials were in aqueous solution.

Materials—*Alkylbenzyldimethylammonium Chlorides*—A homologous series of alkylbenzyldimethylammonium chlorides in the monohydrated form (C_{12}–C_{16}) was used[1]. The purity of these compounds was established by the observation that paper chromatography yielded a single spot. This determination was carried out by other workers (using the same batches) and reported previously (2). Certain physical and interfacial properties of this series were presented in an earlier paper (19).

Cephalin—Synthetic L-α-(β,γ-dipalmitoyl)phosphatidylethanolamine[2] was used as received. The cephalin was dissolved in distilled water for the conductometric studies.

Water—The water used was double distilled from an all-glass apparatus[3].

Methods—A conductivity bridge[4] was used to establish the presence of an interaction between cephalin and the C_{14} and C_{16} homologs. Measurements of the specific conductivity *versus:* (*a*) increasing molar concentration of cephalin in the presence and absence of alkylbenzyldimethylammonium chloride and (*b*) increasing molar concentrations of alkylbenzyldimethylammonium chloride in the presence and absence of cephalin were determined. Corrections were applied for the specific conductivity of the distilled water.

[1] Supplied by Dr. R. A. Cutler, Sterling-Winthrop Research Institute, Rensselaer, N. Y.
[2] California Corporation for Biochemical Research.
[3] Barnstead Redistiller, EPR-½C.
[4] Beckman Instruments, Inc., model RC-19.

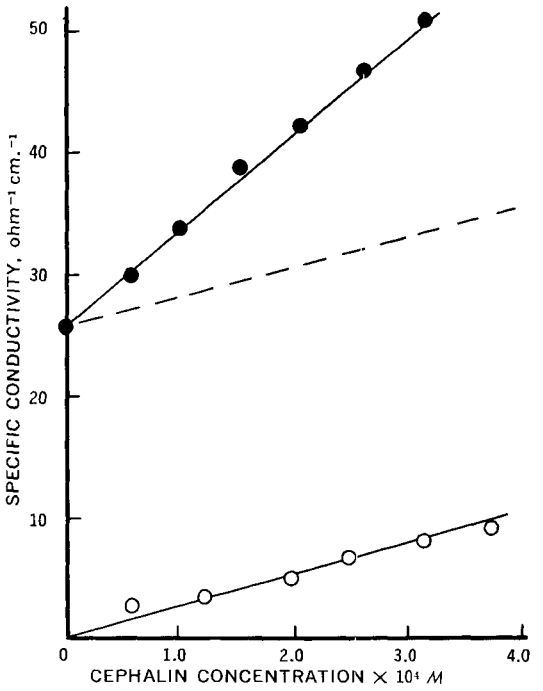

Figure 1—*Conductometric study of increasing concentration of cephalin in the presence and absence of the C_{14} alkylbenzyldimethylammonium chloride. Key:* ○, *no C_{14} alkylbenzyldimethylammonium chloride present; and* ●, *C_{14} alkylbenzyldimethylammonium chloride concentration = 2.51 × 10^{-4} M. Dashed line represents the theoretical additive values.*

RESULTS AND DISCUSSION

Due to the instability of cephalin monolayers in the presence of alkylbenzyldimethylammonium chloride injected into the subphase, a quantitative study of the interaction between these two materials could not be carried out using monolayer techniques. Accordingly, a conductometric study was performed to study their interaction.

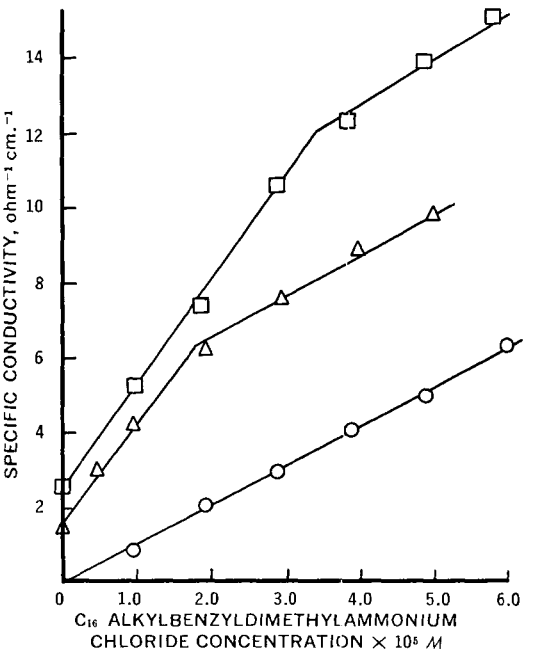

Figure 2—*Conductometric study of increasing concentration of C_{16} alkylbenzyldimethylammonium chloride in the presence and absence of cephalin. Key:* ○, *no cephalin present;* △, *cephalin concentration = 3.43 × 10^{-5} M; and* □, *cephalin concentration = 6.86 × 10^{-5} M.*

Table I—Summary of Conductometric Study of Interaction of Cephalin and Alkylbenzyldimethylammonium Chloride

Homolog	Concentration of Alkylbenzyldimethylammonium Chloride, moles/l.	Concentration of Cephalin, moles/l.	Slope, $ohm^{-1} cm.^{-1} mole^{-1} l.$	Intersection, moles/l. Alkylbenzyldimethylammonium Chloride
14	0	Varying	2.36×10^{-2}	—
	2.51×10^{-4}	Varying	8.21×10^{-2}	—
14	Varying	0	1.07×10^{-1}	—
	Varying	3.13×10^{-5}	2.14×10^{-1} (Initial region)	1.60×10^{-5}
	Varying	3.13×10^{-5}	1.12×10^{-1} (Second region)	
	Varying	6.25×10^{-5}	2.18×10^{-1} (Initial region)	3.50×10^{-5}
	Varying	6.25×10^{-5}	1.10×10^{-1} (Second region)	
16	Varying	0	1.10×10^{-1}	—
	Varying	3.43×10^{-5}	2.81×10^{-1} (Initial region)	1.60×10^{-5}
	Varying	3.43×10^{-5}	1.20×10^{-1} (Second region)	
	Varying	6.86×10^{-5}	2.69×10^{-1} (Initial region)	3.40×10^{-5}
	Varying	6.86×10^{-5}	1.25×10^{-1} (Second region)	

Typical data are shown in Figs. 1 and 2; a summary is presented in Table I.

Figure 1 shows the specific conductivities resulting from the addition of increasing amounts of cephalin to a constant concentration of the C_{14} alkylbenzyldimethylammonium chloride homolog. Since the slope of the mixed system differs from the sum of the single components in solution, it appears that there is an interaction between them in solution. Thus, the slope of the line in Fig. 1 for cephalin in the presence of C_{14} alkylbenzyldimethylammonium chloride is greater than that for cephalin in the absence of the C_{14} homolog. Similarly, as shown in Fig. 2 for the C_{16} homolog, the initial slopes of the lines in the presence of a constant amount of cephalin are greater than those obtained in its absence. Again, these results indicate that an interaction has occurred between the cephalin and alkylbenzyldimethylammonium chloride, leading to specific conductivity for the solution that is increased over that of the separate parts.

As may be reasoned from Fig. 2, as more alkylbenzyldimethylammonium chloride is added to the cephalin, the latter is used to form the interaction product. When no further cephalin is available, the addition of more alkylbenzyldimethylammonium chloride results in a line whose slope is identical to that for alkylbenzyldimethylammonium chloride in the absence of cephalin. Within the limits of experimental error, this appears to be the case. While the amount of data is not felt to be sufficient to propose a mechanism for the observed increase in conductivity, it is possible to gain an appreciation of the stoichiometry of the reaction. Thus, from the points of intersection in Fig. 2, the ratio of cephalin to C_{14} alkylbenzyldimethylammonium chloride is within the range of 1.8–2.0. With the C_{16} alkylbenzyldimethylammonium chloride homolog, the cephalin–alkylbenzyldimethylammonium chloride ratio is from 2.0 to 2.1.

The significance of this interaction in terms of rationalizing the effect of cephalin on the antibacterial activity of alkylbenzyldimethylammonium chloride will be considered in more detail in a subsequent publication (20).

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