The Action of Polyene Antibiotics on Bilayer Lipid Membranes

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Numerous studies on fungi, protozoa, and erythrocytes have shown that the polyene antibiotics act on the cell membrane and limit its ability to function as a selective restraining barrier. These investigations have also indicated that the selective toxicity of the polyene antibiotics is due to interaction with the sterol present in the cell membrane of sensitive organisms. Thus, bacteria and blue-green algae, which lack sterols, are completely unaffected by high concentrations of the antibiotics. The role of sterols in confering polyene sensitivity was clearly demonstrated in experiments with the pleuropneumonia-like organism, Mycoplasma laidlawii. Weber and Kinsky (1965) and Feingold (1965) reported that filipin and amphotericin B had no effect on cells grown in medium devoid of sterols. However, the antibiotics inhibited growth, and caused lysis, of cells that had been cultured in the presence of cholesterol under conditions which lead to the incorporation of the sterol into the cell membrane.

To understand the mechanism by which the polyenes produce such marked permeability alterations, we have examined the effects of these antibiotics on model membrane systems. In an earlier study, it was shown that the polyene antibiotics interact preferentially with lipid monomolecular layers containing sterols (Demel, Kinsky, and van Deenen, 1965). The data were consistent with the hypothesis that the polyenes induce a spatial reorientation of the sterol

molecules and that this rearrangement produces an altered lipid structure within the cell membrane, perhaps, a lamellar to micellar phase transition. It is generally recognized, however, that reactions occuring at an air/water interface are not necessarily applicable to natural membranes. Recently, several laboratories have described the preparation of stable bilayer lipid films separating 2 aqueous compartments (Mueller et al, 1962 a,b; 1963; 1964; Mueller and Rudin, 1963; Huang, Wheeldon, and Thompson, 1964; Huang and Thompson, 1965; Haydon and Taylor, 1963; Hanai, Haydon, and Taylor, 1964, 1965 a,b). These model membranes possess certain dimensional, mechanical, electrical, and permeability properties characteristic of natural cell membranes and, accordingly, the effect of polyene antibiotics on the stability of the bilayer films was examined.

Materials and Methods

Filipin™ was kindly provided by Dr. G.M. Savage, The Upjohn Co., Kalamazoo, Michigan. Nystatin was generously supplied by Dr. D. Perlman, The Squibb Institute for Medical Research, New Brunswick, New Jersey. Perhydrofilipin was prepared by catalytic (PtO_2) hydrogenation in methanol; the product was subsequently purified by preparative thin layer chromatography. Stock solutions of the antibiotics (2.5×10^{-3}M) were made with dimethylformamide:water (57:100).

The thermostated cell and optical system was essentially similar to the apparatus described by Thompson (1964). The chambers each contained 8 ml of 0.1 M NaCl and were separated by a Teflon septum with a 1 mm diameter hole. The bilayer membranes were prepared from solutions of lipid in decane of the following composition: (a) 1% egg lecithin, (b) 1% egg lecithin and 0.5% cholesterol, (c) 1% egg lecithin and 0.05% cholesterol. Assuming a molecular weight of 800 for the chromatographically pure egg lecithin, the molar ratio of lecithin:cholesterol in solutions (b) and (c) was 1:1 and 10:1, respectively. Secondary black films appeared within 3 minutes when systems containing lecithin alone or lecithin:cholesterol (molar ratio, 10:1) were employed; 20 minutes were required when the bilayer was prepared from a solution containing equimolar

amounts of lecithin and cholesterol. In general, the films remained stable for several hours. The experiments were initiated by the introduction of 0.12 ml of the antibiotic solution to one of the chambers to give a final concentration of approximately 4×10^{-5}M. The films were kept under continuous observation for at least 30 minutes and thereafter examined at periodic intervals. All experiments were carried out at 25°C.

Results and Discussion

Table 1 summarizes the principle results of the present investigation. Filipin and nystatin had no effect on the stability of bilayers containing only lecithin. However, films prepared from an equimolar solution of lecithin and cholesterol were disrupted shortly after addition of the antibiotics. These observations are in accord with previous experiments which demonstrated an absence of significant interaction between the polyene antibiotics and monolayers of pure synthetic phospholipids unless sterol was added (Demel, Kinsky, and van Deenen, 1965).

Table 1. Effect of Polyene Antibiotics on the Stability of Lipid Bilayers

Compostion of Solution Used for Membrane Preparation	Addition	Number of Expts.	Average Survival Time of Film (minutes)*
Lecithin	None	8	>60
	Filipin	4	>60
	Nystatin	3	>60
	Perhydrofilipin	3	>60
Lecithin:Cholesterol (1:1 molar ratio)	None	5	>60
	Filipin	11	5½ (2 - 8)
	Nystatin	7	25 (20 - 31)
	Perhydrofilipin	3	>60
Lecithin:Cholesterol (10:1 molar ratio)	None	4	>60
	Filipin	4	17 (14½ - 19)
	Filipin	4	>60**

* Values in parentheses indicate the range.
** See text for discussion.

Investigations with Neurospora protoplasts, mammalian erythrocytes, and Saccharomyces cells (see e.g., Cirillo, Harsch, and Lampen, 1964) have shown that filipin causes more extensive damage to the cell membrane than does nystatin. Filipin has been shown to produce a greater increase in the surface pressure of cholesterol or ergosterol monolayers than nystatin (Demel, Kinsky, and van Deenen, 1965). It therefore seems significant that the bilayers were also less stable (i.e. had shorter survival times) in the presence of filipin than in the presence of nystatin.

These results provide additional support for the contention that the localization of sterol in a membrane is a necessary prerequisite for polyene sensitivity. Experiments with certain specialized subcellular membrane systems, such as mitochondria, suggest however that the presence of sterol per se is not a sufficient condition (Kinsky, Gronau, and Weber, 1965). These studies indicate that the relative amounts of sterols and other lipids, particularly the phospholipid:sterol ratio, may be a factor which determines whether or not a membrane is sensitive to the polyenes. This conclusion is supported by the observation that a 10 fold increase in the lecithin:cholesterol ratio results in the formation of bilayers with longer survival times in the presence of filipin.[1] Some interesting differences in film behavior were noted in this experiment. In 4 trials, the bilayers remained stable for at least 1 hour in the presence of filipin whereas 4 other films, also prepared from a lipid solution with a 10:1 molar ratio of lecithin:cholesterol, had an average survival time of 17 minutes. The reason for this difference is not known but may reflect variation in the lipid composition of the bilayer. Thompson (1964) has previously emphasized the possibility that the relative proportion of lipids in the bilayer may not be exactly identical with the composition of the solution used to prepare the film.

Of particular interest are the results obtained with perhydrofilipin. This derivative, which lacks the conjugated double bond system characteristic of the polyene antibiotics, has approximately 1/100 - 1/500 the potency of

filipin to lyse mammalian erythrocytes. Table 1 shows that perhydrofilipin is without effect on bilayer films which are rapidly disrupted by the polyene antibiotics.[1]

On the basis of the above results, we conclude that the bilayer membranes constitute an extremely appropriate system for the study of the molecular basis of polyene antibiotic action.[2] Conversely, investigations on the mode of polyene action may provide some pertinent information on the structure of natural membranes and the role of sterols. If, in fact, one accepts the prevailing evidence obtained in other laboratories (see introduction) that these model membranes have the dimensions of a bimolecular lipid film, then the marked parallelism between the response of these systems and mammalian erythrocytes, fungal protoplasts, etc., to the polyene antibiotics also suggests that at least some portion of the cell membrane surrounding these structures has a bilayer configuration.

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[1] Additional experiments with monomolecular layers indicate that (a) the increase in surface pressure obtained with filipin depends on the phospholipid: sterol ratio, and (b) perhydrofilipin does not interact as strongly with cholesterol monolayers as filipin (Demel, Crombag, and van Deenen, manuscript in preparation). These results are consistent with the known effects of these compounds on natural membrane systems and bilayers as described in this communication.

[2] It should be noted that in the present experiments the final antibiotic concentration was approximately 4×10^{-5}M. In the case of filipin, this concentration is 20 times the amount usually required to induce the rapid lysis of erythrocytes and 5000 times the amount necessary to produce an increase in the surface pressure of monomolecular layers containing sterols. It seems likely that an appreciable portion of the filipin added to the chamber has interacted with the excess lipid localized at the circumference of the septal orifice. The possibility that lower concentrations of the antibiotics may affect the permeability, electrical, and other properties of the bilayer, without causing a visible destruction of the film, is now under investigation.

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