MECHANISM OF STEROL AND LIPID ANTAGONISM OF A POLYPEPTIDE ANTIBIOTIC, MYCOBACILLIN

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Cholesterol and lecithin can antagonize growth inhibition of *Aspergillus niger* by mycobacillin only if added at the same time as the antibiotic. Experiments with different derivatives of cholesterol and components of lecithin indicate that the 3-β-hydroxyl group of the former and the oleic acid component of the latter are responsible for antagonistic action.

It has been reported that the action of polyene antibiotics is antagonized by sterols[1,2] and phospholipids[3,4,5]. Experiments by WEISSMANN and SESSA[6] with artificial phospholipid spherules with and without cholesterol support the 'sterol action' hypothesis of KINSKY[7] and LAMPEN[2] for polyene antibiotics. Antagonism of the antifungal action of pyrrolnitrin by lipid has also been reported to be caused by physico-chemical interaction.[8] Cholesterol antagonizes the action of mycobacillin, a polypeptide, on *Candida albicans*.[9] Antagonism bas been observed not only by commercial cholesterol and lecithin but also by those isolated from a sensitive strain of *Aspergillus niger*[10]. In this communication we describe some experimental evidences in favour of inactive complex formation to account for the observed antagonistic action of sterol and lipid on mycobacillin.

Materials and Methods

Aspergillus niger was used as test organism and maintained on CZAPEK agar medium. Growth inhibition was measured by standard cup-plate method using a 5-day-old spore suspension as inoculum. Beckmann DU spectrophotometer was used for spectrophotometric measurements. Electrophoresis was carried out in 0.05 M tris buffer (pH 8.6) for 4 hours at 250 V. Mycobacillin and all other compounds were used in alcoholic solution.

Results

Effect of Addition of Cholesterol and Lecithin on the Growth Inhibiting Property of Mycobacillin

When cholesterol in the antagonizing concentration is added to the medium along with the mycobacillin, the inhibitory property of the antibiotic is completely lost. But cholesterol cannot antagonize the inhibition if added separately even 1 hour after the antibiotic. Lecithin behaves in exactly the same way as cholesterol. Thus the antagonistic action of cholesterol or lecithin is exerted only if they are added at 0 hour.

Table 1. Growth antagonism by cholesterol and its derivatives

Antagonist or its derivatives	Concentration in μg/ml	Diameter of zone of inhibition in mm
None	0	23
Cholesterol	125	0
	62.5	23
Cholesterol acetate	500	23
Cholesterol benzoate	500	23
Dihydrocholesterol	125	0
	62.5	23

'0' indicates absence of zone of inhibition. Each cup contains 0.05 ml of mycobacillin (250 μg/ml) alone or the mixture of mycobacillin (250 μg/ml) and the antagonist or any of the derivatives at varying concentrations.

Growth Antagonism by Different Derivatives of Cholesterol

Table 1 shows that cholesterol acetate and cholesterol benzoate do not antagonize the action of mycobacillin at the highest concentration tested, whereas dihydrocholesterol does so in the same concentration as cholesterol itself.

Spectral Behaviour of Mycobacillin in Presence of Cholesterol, Digitonin and Lecithin

Mycobacillin shows an absorption maximum at 277 mμ (Fig. 1). In presence of cholesterol in antagonizing concentration the absorption peak is retained but becomes less prominent. Digitonin when added to the system restores the original nature of the peak. Lecithin does not alter the absorption maximum of mycobacillin (Fig. 2).

Growth Antagonism by Different Components of Lecithin

It appears from Table 2 that from among the different components of lecithin that were tested

Fig. 1. Spectral behaviour of mycobacillin in presence of cholesterol and digitonin
(A) ●-● mycobacillin
(B) ▲-▲ mycobacillin and cholesterol (4: 1)
(C) △-△ mycobacillin and cholesterol (2: 1)
(D) ◇-◇ cholesterol
(E) ○-○ mycobacillin, cholesterol and digitonin

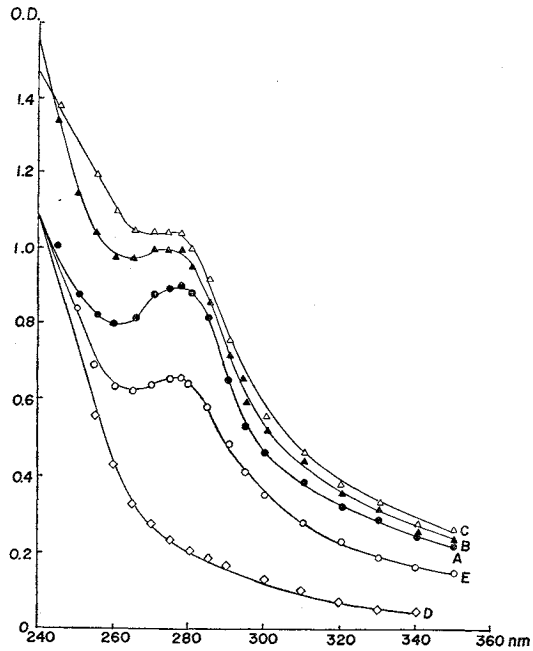


Fig. 2. Spectral behaviour of mycobacillin in presence of lecithin
(A) ●-● mycobacillin
(B) ○-○ mycobacillin and lecithin (4: 1)
(C) ▲-▲ lecithin
(D) △-△ mycobacillin and lecithin (2: 1)

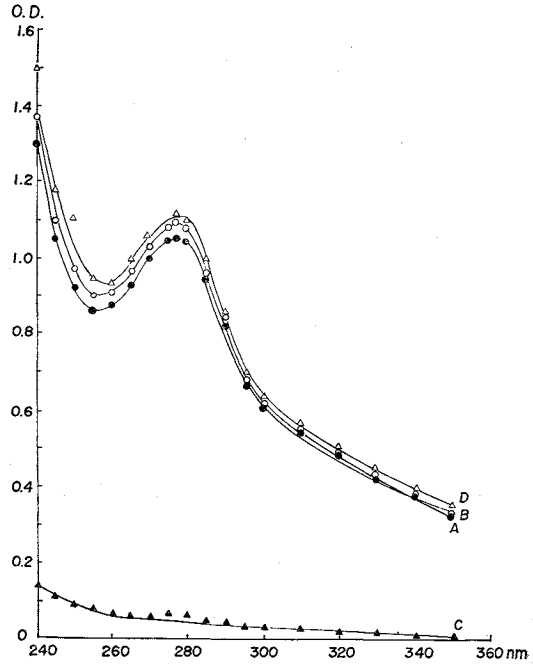


Table 2. Growth antagonism by lysolecithin, lecithin and its different components

Antagonist or its components	Concentration in μg/ml	Diameter of zone of inhibition in mm
None	0	23
Lecithin	125	0
	62.5	23
Choline	500	22
Stearic acid	500	22
Oleic acid	500	0
	250	0
	125	23
	62.5	23
Palmitic acid	500	22
Lysolecithin	500	21
Dipalmitoyl phosphatidyl choline (synthetic lecithin)	500	20

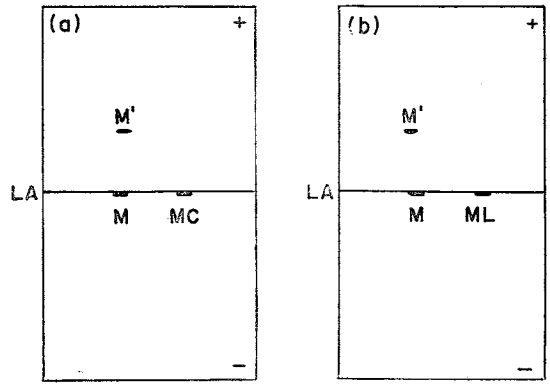
Each cup contains 0.05 ml of mycobacillin (250 μg/ml) alone or the mixture of mycobacillin (250 μg/ml) and component of lecithin in varying concentration. '0' indicates absence of zone of inhibition.

Fig. 3. Electrophoretic mobility of mycobacillin alone and in presence of (*a*) cholesterol or (*b*) lecithin

LA: Line of application

M': Spot of mycobacillin after 4 hours

M: Mycobacillin. MC-mycobacillin and cholesterol (in excess of antagonizing concentration). ML-mycobacillin and lecithin (in excess of antagonizing concentration)



only oleic acid antagonized mycobacillin. Choline, stearic acid, palmitic acid, lysolecithin and dipalmitoyl phosphatidyl choline were inactive.

Electrophoretic Mobility of Mycobacillin alone and in Mixture with Cholesterol or Lecithin

It appears from Fig. 3 that mycobacillin moves through 5 cm from the point of application towards the anode during 4 hours, whereas in case of the mixture of mycobacillin and cholesterol or lecithin (both well in excess of antagonizing concentration) there is no movement. Even at the points of application of the mixture (MC or ML) the presence of mycobacillin could not be detected by its characteristic colour.

Discussion

The present work seeks to study the mechanism of sterol and lipid antagonism of a polypeptide antibiotic mycobacillin. It is suggested that antagonism may occur by either of two possible ways: (1) antibiotic may be inactivated as a result of formation of an inactive complex between the antagonist and the antibiotic itself or (2) the antibiotic may inhibit the biosynthesis of sterols or lipids which when added restore growth. The effect produced by addition of cholesterol and lecithin on the growth inhibition by mycobacillin indicates that the antibiotic might be inactivated due to the formation of an inactive complex with cholesterol or lecithin. Electrophoretic behaviour of mycobacillin alone and in presence of excess of cholesterol or lecithin (to exhaust mycobacillin completely) supports the antibiotic-lipid (sterol or phosphatide) complex formation. Spectra of mycobacillin alone and in the presence of cholesterol or lecithin (Figs. 1 and 2) do not rule out complex formation theory. The lecithin micelle may not effectively react with the chromophore of the antibiotic. It appears from the biological antagonism experiments that the presence of a double bond is not a factor in ensuring the property of cholesterol as an antagonist for which 3-β-hydroxyl group must be kept free. In case of antagonism by lecithin, oleic acid is an essential component,

since synthetic dipalmitoyl phosphatidyl choline which does not contain oleic acid fails to antagonize.

To summarize, the antimycobacillin action of lipid and sterol may owe its origin to lipid and sterol interaction with the antibiotic although the supporting evidence is not very conclusive.

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