Biochimica et Biophysica Acta, 552 (1979) 558–562

BBA Report

BBA 71383

INTERACTIONS BETWEEN BACTERIAL MEMBRANES AND PEPTIDOLIPIDS: LYSIS OF *MICROCOCCUS LUTEUS* PROTOPLASTS BY DERIVATIVES OF PEPTIDOLIPIDIC ANTIBIOTICS FROM *BACILLUS SUBTILIS*

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(Received January 24th, 1979)

Key words: Antibiotic; Peptidolipids, Membrane-peptidolipid interaction; (Micrococcus luteus protoplasts)

Summary

The lysis of protoplasts of *Micrococcus luteus* has been tested with various derivatives of three peptidolipidic antibiotics: iturin A, mycosubtilin and bacillomycin L. The lytic activity is dependent to the nature of the substituting group and to the position of the substituted aminoacid residue. The acetylation of OH groups leads to a decrease of the lytic activity of the natural antibiotics. The methylation of aspartyl residues of bacillomycin L gives a strong lytic activity while natural bacillomycin L has no lytic activity. The methylation of the tyrosyl residue enhances the lytic activities of iturin A and of bacillomycin L-dimethyl ester and reduces that of mycosubtilin.

Correlations between the structures of derivatives and their lytic action on *M. luteus* protoplasts are discussed.

Three peptidolipidic antibiotics have been isolated from various strains of *Bacillus subtilis*: iturin A [1], mycosubtilin [2] and bacillomycin L [3]; their structures have been recently determined [4,5,6]. All these antibiotics belong to the iturin group characterized by a liposoluble β-amino acid with 14 to 17 carbon atoms [7] included in a macrocyclic peptide structure with D and L α-amino acids.

The antibiotic activity is restricted to a few bacteria as *Micrococcus luteus* but is stronger on a large variety of fungi and yeasts. With a view to investigate the correlations between their structure and their biological activity, several derivatives of iturin A, mycosubtilin and bacillomycin L have

been prepared by methyl and acetyl substitution of alcohol and phenol groups and by methylation of carboxyl groups in the side chains of amino acids. The antibacterial activities of these derivatives were tested on *M. luteus* and they were found smaller than those of natural antibiotics [10].

From the peptidolipidic structure of the iturin group of antibiotics one could assume an interaction with the cytoplasmic membrane of microorganisms. This interaction had been studied by measuring the lytic activity of iturin A, mycosubtilin and bacillomycin L on the protoplasts of *M. luteus*. Iturin A and mycosubtilin had a lytic activity on these protoplasts while bacillomycin L had no lytic activity [8, 9]. As these antibiotics differ essentially in their amino acids, there is a correlation between the interaction with cytoplasmic membrane and the structure of the peptidic moiety of the antibiotics. To clarify the role of polar groups of the peptidic part in this interaction we have studied the lytic activity of the methyl and acetyl derivatives of iturin A, mycosubtilin and bacillomycin L on the protoplasts of *M. luteus*.

Antibiotics and their derivatives were obtained as previously described [10]. Fig. 1 shows the formula of the compounds which have been tested.

M. luteus NCTC 2665 was grown at 35°C on a brain/heart medium, 37 g/l (Bio-Merieux, France) for 15 h. After harvesting the bacteria by cen-

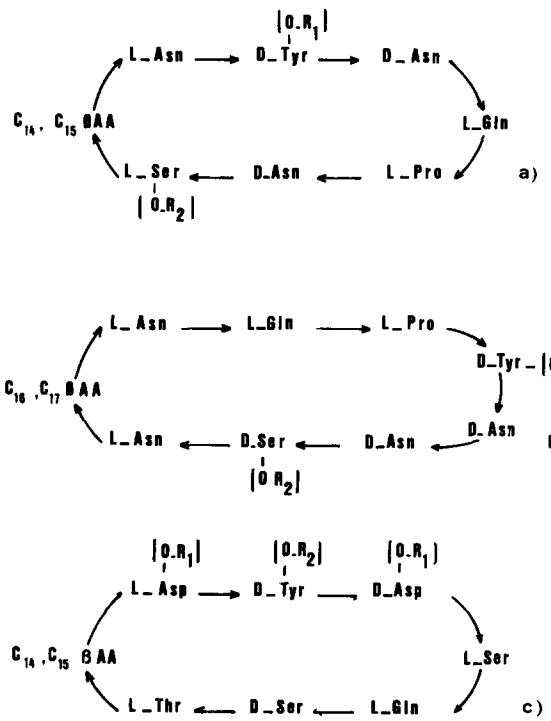

Fig. 1. Structure of three peptidolipidic antibiotics. (a) Iturin A: $R_1 = R_2 = H$; compound I : $R_1 = CH_3$, $R_2 = H$; compound II : $R_1 = COCH_3$, $R_2 = H$; compound III : $R_1 = H$, $R_2 = COCH_3$; compound IV : $R_1 = R_2 = COCH_3$. (b) Mycosubtilin: $R_1 = R_2 = H$; compound V : $R_1 = CH_3$, $R_2 = H$; compound VI : $R_1 = COCH_3$, $R_2 = H$; compound VII : $R_1 = H$, $R_2 = COCH_3$; compound VIII : $R_1 = R_2 = COCH_3$. (c) Bacillomycin L : $R_1 = R_2 = H$; compound IX : $R_1 = CH_3$, $R_2 = H$; compound X : $R_1 = R_2 = CH_3$; compound XI : $R_1 = H$, $R_2 = CH_3$.

trifugation, protoplasts were obtained from the lysozyme-treated cells in a Tris-HCl/sucrose buffer, pH 8.0 as in [8]. The lytic activity of the compounds was tested on a suspension of protoplasts in a solution 20% sucrose in 20 mM Tris-HCl buffer, initial absorbance = 0.35. The kinetics of lysis of protoplasts after adding various concentrations of antibiotic derivatives was followed by plotting the absorbance with a Beckman spectrophotometer connected to a recorder. All the compounds gave curves of the same type as is shown in Fig. 2 for the *O*-methyltyrosine derivative of iturin A (compound I) at 20, 60, 100 μg/ml concentrations.

When lysis occurred, the absorbance decreased very quickly and remained roughly constant after 5 min. The absorbance values at this time were used for the calculation of the percentage of lysis of protoplasts.

The lytic activities of the derivatives of iturin A, mycosubtilin and bacillomycin L are reported in Table I. In addition to the activities of *O*-methyl and *O*-acetyl derivatives, Table I shows the data obtained with iturin C a 'companion' of iturin A which has an L-aspartyl residue instead of an L-asparaginyl residue linked to the carboxylic group of the β-amino acid [11].

No lytic activity is found with iturin C, bacillomycin L and its *O*-methyltyrosine derivative (compound XI); other compounds have lytic activities varying with the structure of derivatives. The following conclusions can be inferred from the results.

(1) The absence of carboxyl groups near the hydrophobic chain of the β-amino acid is a very strict condition for lytic activity on *M. luteus* protoplasts. This hypothesis which was suggested previously [9] is strongly confirmed by the present work. Bacillomycin L and iturin C which have an

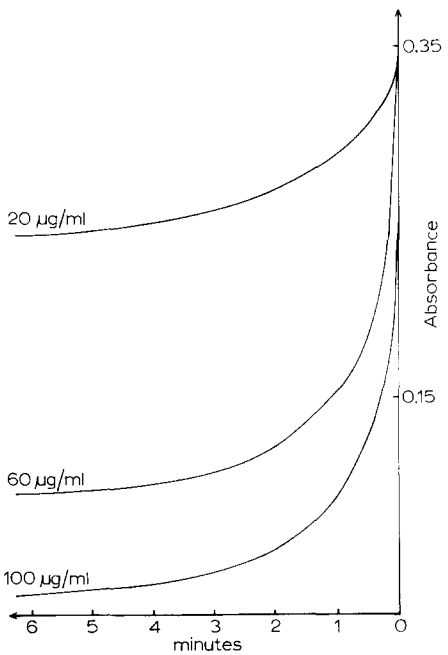

Fig. 2. Kinetics of lysis of *M. luteus* protoplasts by various concentrations of O-methyltyrosine-iturin A (compound I).

TABLE I

Percentages of lysis of *Micrococcus luteus* protoplasts by iturin A, mycosubtilin, bacillomycin L and their derivatives

Concentration (μg/ml)	10	20	50	100	200
Iturin A	3	6	10	30	60
O-Methyltyrosine-iturin A (compound I)	16	32	78	95	
Iturin C	0	0	0	0	0
Acetyltyrosine-iturin A (compound II)	5	8	20	29	46
Acetylserine-iturin A (compound III)	5	8	18	19	19
Diacetyliturin A (compound IV)	5	8	18	20	20
Mycosubtilin	80	92			
O-Methyltyrosine-mycosubtilin (compound V)	38	63	70	68	70
Acetyltyrosine-mycosubtilin (compound VI)	7	10	27	37	48
Acetylserine-mycosubtilin (compound VII)	7	11	30	44	57
Diacetylmycosubtilin (compound VIII)	7	11	30	46	76
Bacillomycin L	0	0	0	0	0
Bacillomycin L dimethylester (compound IX)	15	49	70	76	76
O-Methyltyrosine-bacillomycin L dimethylester (compound X)	35	69	95		
O-Methyltyrosine-bacillomycin L (compound XI)	0	0	0	0	10

aspartyl residue linked to the carboxyl group of the β-amino acid give no lysis of protoplasts. When the aspartyl residues of bacillomycin L are esterified (compound IX) a strong lytic activity appears; if only the tyrosyl residue of bacillomycin L is methylated (compound XI), there is no significant modification of the lack of activity of the antibiotic.

(2) *O*-Methylation of the tyrosyl residue of active compounds has either a positive or a negative effect according to the position of this residue in the antibiotic. The lytic action of iturin A and of bacillomycin L dimethyl ester (compound IX) is enhanced when the tyrosyl residue is methylated (compounds I and X) while the *O*-methyltyrosine derivative of mycosubtilin (compound V) has a smaller lytic activity than mycosubtilin. A comparison of the structures of the three antibiotics (Fig. 1) could give an explanation to these results. In iturin A and in bacillomycin L the tyrosyl residue is located in the second position from the lipophilic β-amino acid and in mycosubtilin the tyrosyl residue is located in the fourth position, in the opposite direction to the β-amino acid. It seems that the lytic activity is greatly enhanced when the polarity near the carboxyl group of the β-amino acid is diminished. On the contrary, the presence of a polar group on the fourth amino acid has a positive effect; thus bacillomycin L dimethyl ester and mycosubtilin, which have an OH group on the fourth amino acid, show a much stronger lytic activity than iturin A which has no polar group on the fourth amino acid. Moreover at a low concentration (20 μg/ml) the compounds whose first three amino acid residues have roughly similar polarities and which differ by the polarity of the fourth amino acid have lytic activities reasonably correlated to this polarity: mycosubtilin with a tyrosyl residue has the strongest lytic activity on protoplasts, *O*-methyltyrosine-bacillomycin L dimethylester (compound X) with a less polar seryl residue has a smaller lytic activity and *O*-methyltyrosine-iturin A (compound I), with a glutaminyl residue is even less active on protoplasts.

(3) The acetylation of the OH groups of iturin A and of mycosubtilin always gave a decrease of the lytic activity. The acetyl derivatives are less polar compounds than natural antibiotics but the acetyl group could introduce a

steric inhibition in the antibiotic-membrane interactions. Recent NMR studies have shown the presence of two hydrogen bonds in iturin A giving a rigid conformation for the peptide cycle [12]. The acetylation of hydroxyl groups could bring steric hindrance in the molecule which could not keep its natural conformation any longer and there is a possible correlation between the lytic activity and the conformation of the molecule.

(4) The most unexpected conclusion is the large discrepancy between the antibacterial activity of antibiotics and of their derivatives and their action on the bacterial cytoplasmic membrane. Recent results [10] have shown a decrease of the antibacterial activity on *M. luteus* of all the antibiotic derivatives. This decrease is not observed in the action on the cytoplasmic membrane of this bacterium. The most striking example is bacillomycin L which has the same antibacterial activity that iturin A and mycosubtilin have while it has no lytic activity on protoplasts of *M. luteus*. The *O*-methyltyrosine-bacillomycin L dimethyl ester (compound X) has a smaller antibacterial activity than bacillomycin L while its lytic action is strongly enhanced. Thus, some of the peptidolipidic antibiotics have strong interactions with the bacterial membrane but this interaction does not explain entirely the antibacterial activity.

This research was supported by the Délégation Générale â la Recherche Scientifique (contrat No. 77.7.0491) and the Centre National de la Recherche Scientifique (A.I. No. 031201). We thank Dr. L. Delcambe, Centre National de Production et d'Etude des Substances d'Origine Microbienne, Liêge, Belgique, who provided us with antibiotics.

References

1 Delcambe, L. (1965) Bull. Soc. Chim. Belg. 74, 315—328
2 Walton, R.B. and Woodruff, H.B. (1949) J. Clin. Invest. 28, 924—926
3 Landy, M., Warren, G.H., Rosenman, S.B. and Colio, L.G. (1948) Proc. Soc. Exp. Biol. Med. 67, 539—541
4 Peypoux, F., Guinand, M., Michel, G., Delcambe, L., Das, B.C. and Lederer, E. (1978) Biochemistry 17, 3992-3996
5 Peypoux, F., Michel, G. and Delcambe, L. (1976) Eur. J. Biochem. 63, 391—398
6 Besson, F., Peypoux, F., Michel, G. and Delcambe, L. (1977) Eur. J. Biochem. 77, 61—67
7 Peypoux, F., Guinand, M., Michel, G., Delcambe, L., Das, B.C., Varenne, P. and Lederer, E. (1973) Tetrahedron 29, 3455—3459
8 Besson, F., Peypoux, F., Michel, G. and Delcambe, L. (1978) Biochem. Biophys. Res. Commun. 81, 297—304
9 Besson, F., Peypoux, F. and Michel, G. (1978) FEBS Lett. 90, 36—40
10 Peypoux, F., Besson, F., Michel, G. and Delcambe, L. (19) J. Antibiotics, in the press
11 Peypoux, F., Besson, F., Michel, G., Delcambe, L. and Das, B.C. (1978) Tetrahedron 34, 1147—1152
12 Garbay-Jaureguiberry, C., Roques, B.P., Delcambe, L., Peypoux, F. and Michel, G. (1978) FEBS Lett. 93, 151—156