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SYNTHETIC FRAGMENTS OF BACTERIAL CELL WALLS. PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES

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Introduction

There are numerous compounds which show an immunoadjuvant and/or immunostimulating effect. Of their number, the fragments of bacterial cell wall, peptidoglycan, MDP, and other peptides and glycopeptides (1) are of key importance since they permit us a direct examination of the relationship between the chemical structure and the biological properties of the components of bacterial cell walls. The character of the peptidoglycan is that of a sequential polymer. We have designed therefore the first part of our study as an examination of the relationship between biological effects and the complexity of synthetic glycopeptides, fragments of the cell wall peptidoglycan of Staphylococcus aureus, strain Copenhagen and Streptococcus pyogenes, group A. The peptides and glycopeptides needed for our study were prepared both by solid phase synthesis and by synthesis in solution. Attention deserves the simultaneous removal of the protecting groups from the glycopeptides by treatment with sodium in liquid ammonia (1,2) and the use of solid phase synthesis for the preparation of glycopeptides (1). In this communication we

have focused a special attention to the problem of the sugar component.

Results

1) HPLC, ^{1}H- and ^{13}C-NMR studies. The high performance liquid chromatography (HPLC-reversed phase) of MDP, galacto-, allo-, and norallo-MDP showed the presence of three components at least, in each case (see Table I). The position of the individual peaks is characterized by the capacity factor k'. In the case of MDP the products represented by the individual peaks were isolated and equilibrated with an aqueous methanolic phosphate buffer. The equilibrium mixture contained pairs of the starting compounds. This result shows that MDP represents a mixture of α and β anomers and two impurities. The HPLC data were confirmed by measurement of ^{1}H- and ^{13}C-NMR spectra. According to these data peak 1 corresponds to the β-anomer and peak 2 to the α-anomer. Analogous results were obtained recently by Lederer et al. (3). Peaks 3 and 4 are impurities with the opposite configuration of the lactyl residue since they yielded isomuramic acid after hydrolysis (identification by HPLC and in the amino acid analyzer). The condensation of the protected glucosamine derivative with the derivative of 2-halogenpropionic acid obviously does not proceed quite stereoselectively (cf., 4). The HPLC of galacto-, allo-, and norallo-MDP indicated a mixture of three compounds (Table I). Each of these components afforded after equilibration, carried out as with MDP, the original equilibrium mixture. We account for two of the peaks by the presence of the α- and β-anomer of the pyranoid form; the third peak corresponds to the furanoid form. This assumption is supported by the results of experiments with the reduction of D-galacto-MDP by sodium

TABLE I

Composition of glycopeptides in phosphate buffer pH 5.00 in equilibrium

compound	No.of peak	k′	% ($t_{eq.}$)	isomer[a] α,β;P,F
MDP	1	4.7	26.8	β - P
	2	8.5	53.6	α - P
	3	9.8	7.0	β - P (S)
	4	15.8	12.6	α - P (S)
D-galacto-MDP	1	4.2	34.1	β - P
	2	6.6	59.7	α - P and β - F
	3	8.1	6.2	α - F
product of $NaBH_4$ reduction of D-galacto-MDP	1	7.7	88.4	
	2	10.4	11.6	
D-norallo-MDP	1	4.9	56.0	β - P
	2	5.9	20.5	α - F
	3	6.5	22.9	α - P
	4	8.0	0.6	β - F
D-allo-MDP	1	7.8	42.6	β - P
	2	8.8	0.0	β - F
	3	10.8	40.8	α - P
	4	13.1	16.6	α - F

[a] P = pyranoid, F = furanoid form

borohydride which afforded one product only. The ^{1}H- and ^{13}C-NMR identification of the products corresponding to the individual peaks is in Table I. The measurement of the kinetics of mutarotation of the α- and β-anomers of MDP showed that an equilibrium is established at 24°C in both cases after approximately the same period (3 hours). At 0°C the rate of equilibration is 1.8×10^{-3} min^{-1}. The rate of equilibration at 0°C for the D-allo-MDP products was the same for the compounds of k′ 7.8 and 10.8 (k 2.5×10^{-3} min^{-1}) whereas a much higher rate was observed

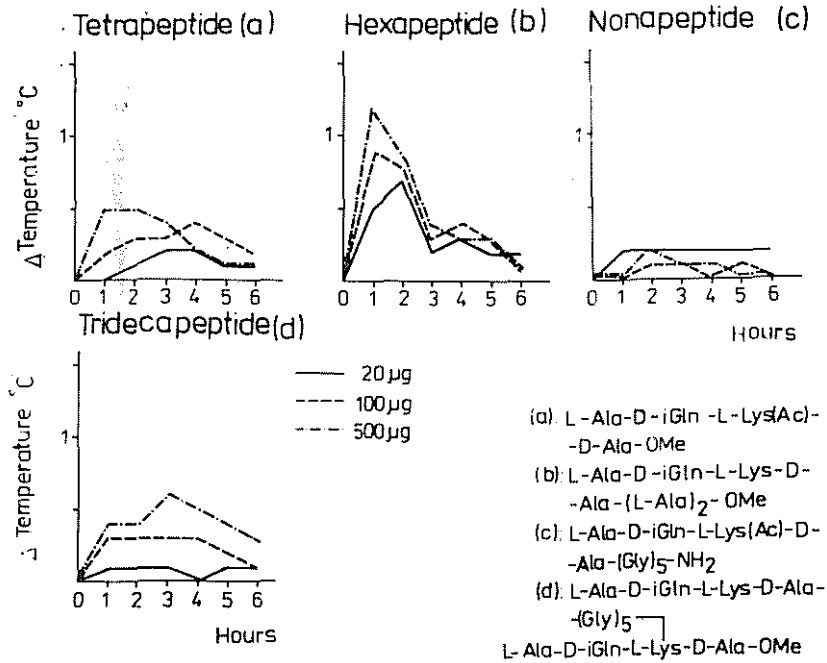

Fig. 1
Pyrogenicities of peptides

with the compound of k′ 13.1 (k 1.4 x 10^{-2} min^{-1}).

2) Biological effects. Multiple biological activities of streptococcus peptidoglycan subunits and analogs have been demonstrated in vivo and in vitro experiments. These activities resemble or are identical with the biological properties of streptococcus peptidoglycan. Our attention was particularly focused on three effects. Pyrogenicity was the first pronounced biological feature studied. The peptidoglycan synthetic subunits were injected intravenously to groups of 3-6 rabbits, each rabbit receiving its dose in a volume of 2 ml. The pyretic responses to doses of 20, 100 and 500 µg were consistent among animals in individual groups. Fig. 1 depicts the mean values of fever response to peptides. With the exception of the hexapeptide, no

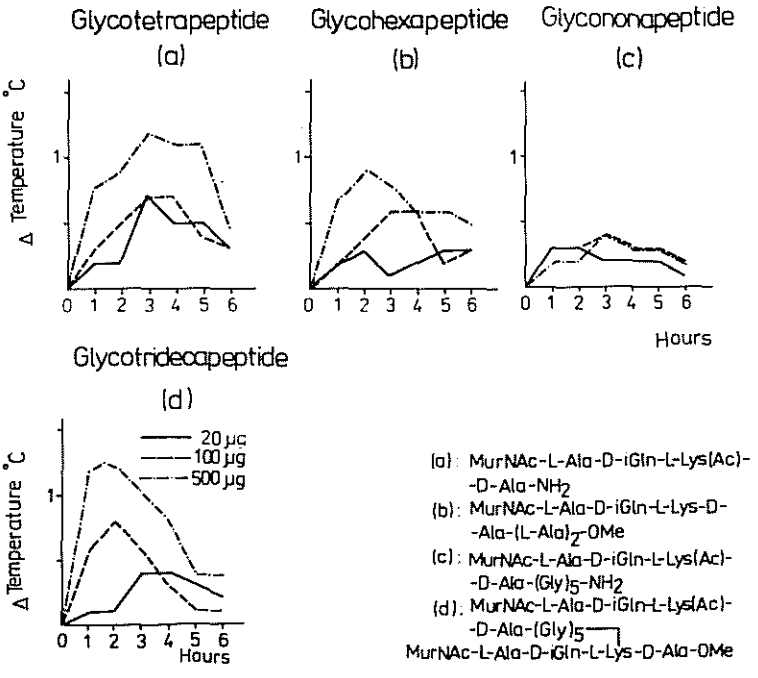

Fig. 2
Pyrogenicities of glycopeptides

clear cut fever effect was recorded for the peptides even after a dose of 500 µg. The prolongation of the chain did not play any evident role. Fig. 2 shows that the attachment of the muramyl residue to the peptide molecule resulted in an evident pyrogenicity in the glycodipeptide, glycohexapeptide, and glycotridecapeptide, whereas the glyconona peptide was without any evident fever effect. The glycodipeptide was evidently the most pyrogenic analog. The immunoadjuvant activity of the synthetic analogs was tested on albino guinea pigs injected either ovalbumin or a hydrochloric acid extract of streptococcus M protein type 24. The glycopeptides injected with the antigens produced a clear delayed hypersensitivity reaction of various degree of intensity. A similar potentiation was

recorded in humoral response as measured by the antibody answer to the antigen. Peptides were with a rare exception without effect. Thrombocytolysis of rabbit blood platelets could be provoked by glycopeptides whereas the peptides themselves had no effect or produced degranulation only. The lysis of blood platelets was dose dependent. In all three effects, in pyrogenicity, immunoadjuvancy and thrombocytolysis, an evident structure to function relationship could be demonstrated.

References

1. Zaoral, M., Ježek, J., Krchňák, V., Straka, R.: Collect. Czech.Chem.Commun. 45, 1424-1446 (1980).
2. Zaoral, M., Ježek, J., Straka, R., Mašek, K.: Collect. Czech.Chem.Commun. 43, 1797-1802 (1978).
3. Halls, T.D.J., Raju, M.S., Wenkert, E., Zuber, M., Lefrancier, P., Lederer, E.: Carbohydr.Res. 81, 173-176 (1980).
4. Arendt, A., Kolodziejczyk, A., Sokolowska, T.: Rocz.Chem. 48, 1707-1711 (1974).