MOENOMYCIN, AN INHIBITOR OF CELL WALL SYNTHESIS

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Received October 9, 1967

The antibiotic moenomycin (Wallhäußer et al., 1965, Huber et al., 1965) which is isolated from Streptomyces ghanaensis ATCC 14,672 and other strains is a complex of several chemically very similar phosphorus-containing compounds. The properties of the recently published antibiotics 11,837 R.P. (Mancy et al., 1966) and prasinomycin (Weisenborn et al., 1967) correspond practically to those of moenomycin.

Moenomycin is mainly effective against gram-positive microorganisms (bacteriostasis). The low toxicity of the antibiotic points to a mode of action selective in respect of bacterial cells. In the present study we have investigated the influence of moenomycin on the cell wall synthesis of Staphylococcus aureus. With S. aureus growing in the presence of sublethal concentrations of penicillin, Park and Johnson (1949) observed an accumulation of uridine nucleotide acetylamino sugar derivatives, especially of the pentapeptide UDP-GNAc-lactyl-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala* (I) (Park, 1952) which was recognized as the precursor of the cell wall peptidoglycan the incorporation of which is inhibited by

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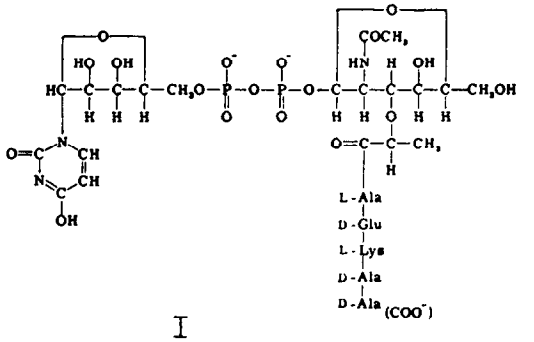
* Abbreviations: GNAc = N-acetylglucosamine
GNAc-lactyl = MurNAc = N-acetylmuramyl

penicillin (Fig. 1). Analogous accumulations of uridine nucleotides characteristic of cell wall synthesis were observed with bacitracin, novobiocin, ristocetin and vancomycin.



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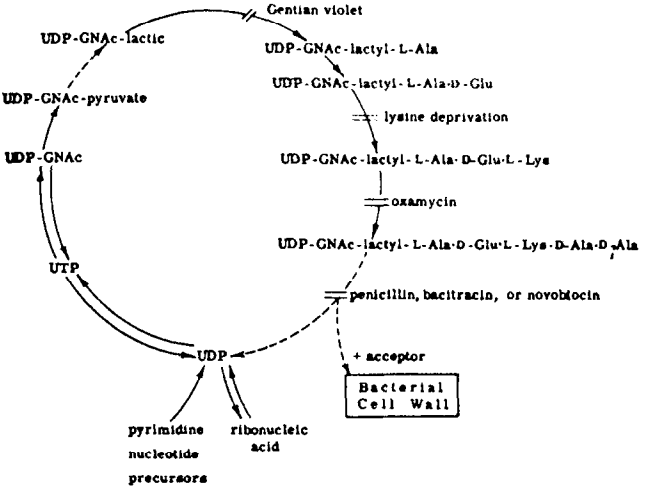

Fig. 1. Biosynthesis of uridine nucleotide precursors of the cell wall peptidoglycan (according to Stromin-ger, 1962).

Our experiments show that staphylococci growing in presence of small doses of moenomycin, accumulate UDP-acetylamino sugars too. After separation by ion exchange the main

component was identified as UDP-MurNAc-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala. The action of moenomycin against staphylococci is thus based on inhibition of the cell wall synthesis of the micro-organism.

Experimental

S. aureus 209P was cultivated according to the method of Park (1952), the progress of growth was followed by turbidimetry. The cultures were prepared in shaking cultures and fermenters. Moenomycin (0.8 mcg/ml) and penicillin (1 mcg/ml) were added at the point of half maximum growth. The cells were harvested 0.5, 1, 2, 3, and 4 hours after the addition.

Extraction of the cells with cold trichloroacetic acid, determination of the N-acetylamino sugars and separation of the nucleotides by anion exchange chromatography using Dowex-1x2 (Cl^-), 200 - 400 mesh, was performed as described by Strominger (1957). Composition of the isolated uridine nucleotide peak was determined as follows: UDP spectrophotometrically at 262 mμ (molar extinction coefficient 10,000), phosphorus according to Werkheiser et al. (1953), labile phosphorus after hydrolysis with 1 N HCl (10 min.), amino acids by means of the Beckman amino acid analyzer after 20 hours' hydrolysis with 6 N HCl, N-acetylhexosamine (calculated as N-acetylglucosamine) according to the method of Reissig et al. (1955).

Results and Discussion

Fig. 2 shows the concentration of the amino sugar-containing

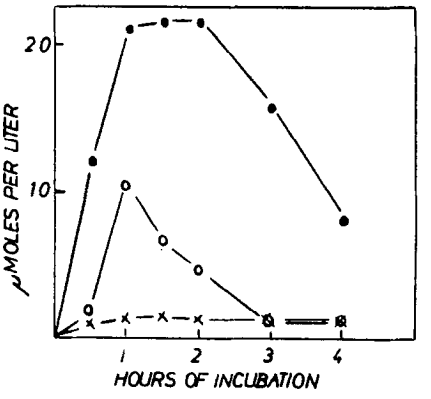

Fig. 2. Time course of accumulation of uridine nucleotides in S. aureus. µM N-acetylhexosamine/l
●——● moenomycin, 0.8 mcg/ml
O——O penicillin G, 1 mcg/ml
×——× control

uridine nucleotides in the course of the growth of S. aureus in presence of moenomycin (0.8 mcg/ml) and penicillin G (1 mcg/ml) compared with the control. The maximum for moenomycin is between one and two hours' incubation (21.6 µM N-acetylglucosamine/liter); for penicillin it is one hour. In our experiments moenomycin is distinctly more effective than penicillin.

For identification the accumulated uridine nucleotides were separated chromatographically on a Dowex-1 anion exchanger (Strominger, 1957) in the course of which UV-absorption and N-acetylhexosamine were determined. In the elution diagram (fig. 3) only peak IV contains hexosamine and an UV-maximum at 262 mµ that points to uridine nucleotides. The other main peaks I (260 mµ), II and III (258 mµ) would seem to be in respect of adenosine nucleotides.

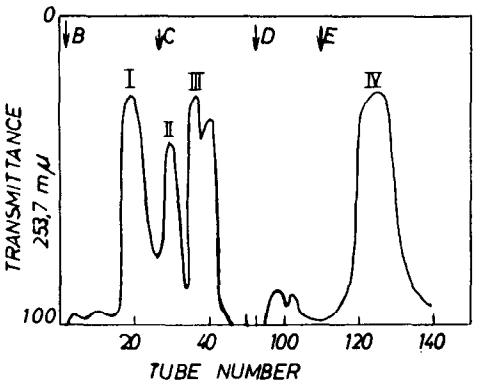

Fig. 3. Ion exchange separation of nucleotides in TCA-extract from moenomycin-treated S. aureus. Dowex-1x2 (Cl^-), 200-400 mesh. Elution according to Strominger (1957): B 0.01 N HCl, C 0.05 M NaCl in 0.01 N HCl, D 0.1 M NaCl in 0.01 N HCl, E 0.1 N HCl.

Table. Analysis of Peak IV

	μmol/ml	mol per mol hexosamine
N-acetylhexosamine	3.28	1.0
UDP	3.36	1.02
P total	6.25	1.98
P labile	3.23	0.98
Glutamic acid	3.25	0.99
Lysine	4.20	1.28
Alanine	9.94	3.03

The analysis of peak IV (Table) shows that per mol of hexosamine there is one mol of UDP, two mols of phosphorus, one mol each of glutamic acid and lysine, as well as three mols of alanine. Although the lysine value is slightly excessive

the analysis result indicates that Park's nucleotide, UDP-MurNAc-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala is concerned which as a precursor of the cell wall peptidoglycan always accumulates in those cases where the biosynthesis of the cell wall is inhibited by antibiotics such as penicillin, bacitracin, novobiocin, ristocetin and vancomycin. The action of moenomycin on S. aureus is therefore also based on an inhibition of the cell wall synthesis in which the incorporation of the UDP-NAc-muramyl-pentapeptide is blocked. The precise point of attack is not yet known. Whether moenomycin like vancomycin and ristocetin inhibits the incorporation of the muramyl-pentapeptide combined with a phospholipid carrier into the cell wall peptidoglycan (Anderson et al., 1967) or like penicillin disturbs cross-linking of it (Strominger et al., 1967) remains to be clarified.

The selectivity of the moenomycin action on a process specific for the micro-organism explains the extraordinarily low toxicity of the antibiotic.

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