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Inhibition of phosphoenolpyruvate:uridine diphosphate *N*-acetylglucosamine enolpyruvyltransferase by uridine diphosphate *N*-acetylmuramyl peptides

P.S. VENKATESWARAN [a], E.J.J. LUGTENBERG [a,b] and H.C. WU [a]

[a] *Department of Microbiology, University of Connecticut Health Center, Farmington, Conn. 06032 (U.S.A.) and* [b] *Laboratory for Microbiology, State University, Catharijnesingel 59, Utrecht (The Netherlands)*

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SUMMARY

Among uridine diphosphate *N*-acetylmuramyl peptides, UDP-*N*-acetylmuramic acid-L-Ala-D-Glu-*meso*-diaminopimelic acid and UDP-*N*-acetylmuramic acid-L-Ala-D-Glu-*meso*-diaminopimelic acid-D-Ala-D-Ala were found to inhibit the activity of phosphoenolpyruvate:uridine diphosphate *N*-acetylglucosamine enolpyruvyltransferases from *Escherichia coli* and from *Bacillus cereus*.

In spite of the fact that the gross chemical structure and the mode of biosynthesis of peptidoglycan in bacteria have been well established[1] very little is known concerning the regulation of the synthesis of this giant bag-shaped macromolecule. It has been known since the pioneering work of Park and Johnson[2] that an inhibition of the biosynthesis of peptido glycan in Gram-positive bacteria such as *Staphylococcus aureus* by penicillin results in the accumulation of nucleotide-sugar precursors of peptidoglycan, especially UDP-*N*-acetylmuramic acid(MurNAc)-pentapeptide (UDPMurNAc-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala)[2,3]. However, accumulation of UDPMurNAc-pentapeptide (UDPMurNAc-L-Ala-D-Glu-*meso*-diaminopimelic acid(Dpm)-D-Ala-D-Ala) is not seen in some Gram-negative bacteria such as *Escherichia coli* in which the synthesis of peptidoglycan has been curtailed by the action of antibiotics such as vancomycin or penicillin[4]. A simple explanation for this lack of accumulation of UDPMurNAc-pentapeptide in *E. coli* cells inhibited in peptidoglycan synthesis, is the operation of an efficient feedback regulatory mechanism on the activity of enzymes involved in the biosynthesis of cell wall precursors (Fig. 1) by UDPMurNAc-pentapeptide.

Abbreviations: MurNAc, *N*-acetylmuramic acid; GlcNAc, *N*-acetylglucosamine; Dpm, diaminopimelic acid.

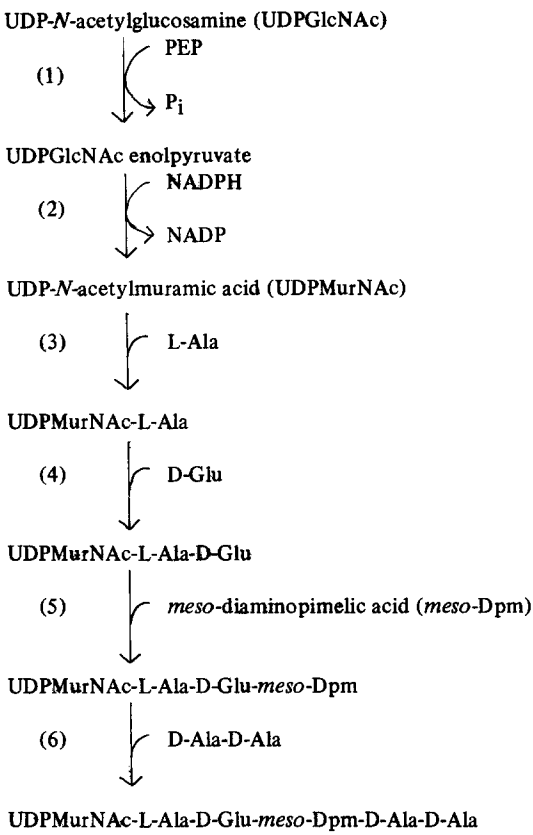


Fig. 1. Pathway for the biosynthesis of UDP-*N*-acetylmuramyl pentapeptide in *E. coli*. Enzymes catalyzing the reactions as numbered above are: (1) PEP:UDPGlcNAc enolpyruvyltransferase. (2) UDPGlcNAc enolpyruvate reductase. (3) UDPMurNAc:L-alanine ligase (ADP) (EC 6.3.2.8) (L-alanine adding enzyme). (4) UDPMurNAc-L-Ala:D-glutamic acid ligase (ADP) (EC 6.3.2.9) (D-glutamic acid adding enzyme). (5) UDP-MurNAc-L-Ala-D-Glu:diaminopimelic acid ligase (ADP) (*meso*-Dpm adding enzyme). (6) UDPMurNAc-L-Ala-D-Glu-*meso*-Dpm:D-Ala-D-Ala ligase (ADP) (D-alanyl-D-alanine adding enzyme).

The most logical target for a feedback inhibition of this complex pathway would seem to be the very first unique enzyme, namely phosphoenolpyruvate(PEP):UDP-*N*-acetyl glucosamine enolpyruvyltransferase. Anwar and his co-workers[5] reported the inhibition of this enzyme from *Enterobacter cloacae* by UDP-*N*-acetylmuramyl-L-Ala-D-Glu-*meso*-Dpm. We wish to report in this communication a study of the inhibition of this enzyme from *E. coli* and from *Bacillus cereus* by various UDP-*N*-acetylmuramyl peptides. We chose to study the enzyme from *B. cereus* since it has been shown previously that in *B. cereus* cells, one can indeed induce by vancomycin treatment an accumulation of UDPMurNAc-pentapeptide[3].

Bacterial strains used for the present study are *E. coli* strain K-235, *E. coli* K-12 strain KMBL-146 and *B. cereus* T[3,4]. The growth media (L broth), conditions for growth

of cells, extraction of crude enzyme, partial purification by $(NH_4)_2SO_4$ precipitation and assay of PEP:UDP-*N*-acetylglucosamine(GlcNAc) enolpyruvyltransferase are the same as described previously[6]. The 40–60% $(NH_4)_2SO_4$ fraction was used as the enzyme in this study. The final volume of the assay mixture was 25 μl with the final concentrations of UDPGlcNAc and PEP being 20 mM and 0.12 mM (3000 cpm/nmole of [^{14}C]PEP), respectively. UDP-*N*-acetylmuramic acid and its derivative peptides were prepared as described earlier[3,4].

Among the various UDP-*N*-acetylmuramyl peptides, tri- and pentapeptides are quite potent inhibitors of phosphoenolpyruvate:UDP-*N*-acetylglucosamine enolpyruvyltransferase from *E. coli* strain K-235 while UDPMurNAc, UDPMurNAc-L-Ala and UDPMurNAc-L-Ala-D-Glu inhibit weakly (Table I). In a control experiment, we found no

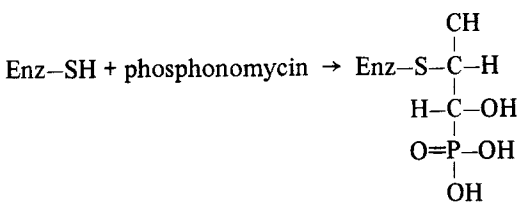
TABLE I

INHIBITION OF PEP:UDPGlcNAc ENOLPYRUVYLTRANSFERASE FROM *E. coli* STRAIN K-235 BY UDP-*N*-ACETYLMURAMYL PEPTIDES

UDPMurNAc or one of its derivatives was added to a final concentration of 5 mM, as indicated. The enzyme was added last. Incubation was carried out at 37 °C for 10 min.

Addition	*Relative enzyme activity*
None	100
UDPMurNAc	64
UDPMurNAc-L-Ala	61
UDPMurNAc-L-Ala-D-Glu	44
UDPMurNAc-L-Ala-D-Glu-*meso*-Dpm	35
UDPMurNAc-L-Ala-D-Glu-*meso*-Dpm-D-Ala-D-Ala	18

inhibition of PEP:UDPGlcNAc enolpyruvyltransferase from *E. coli* by UDPgalactose at a final concentration of 2 mM. The extent of inhibition of this enzyme by UDPMurNAc-peptides could be enhanced by preincubation of the enzyme with the effectors, especially with UDP-*N*-acetylmuramyl tripeptide (90% inhibition by 5 mM tripeptide). The enhancement of inhibition of this enzyme by preincubation with the effector could be prevented by the addition of dithiothreitol (2 mM). This result suggests that in the absence of dithiothreitol the enzyme undergoes inactivation as a result of the exposure and auto-oxidation of one or more critical sulfhydryl groups possibly in or near the active site of this enzyme. Kahan, F. and his co-workers (personal communication) have postulated that the irreversible inactivation of this enzyme by phosphonomycin (L-*cis*-2-epoxypropylphosphonic acid) most likely results from a reaction (i) analogous to the enzyme–substrate (PEP) intermediate in catalysis (ii):



(i)

$$\text{Enz–SH} + \text{PEP} \rightleftarrows \text{Enz–S–C}(\text{COOH})=\text{CH}_2 + \text{P}_i \qquad \text{(ii)}$$

This irreversible inhibition by phosphonomycin requires the presence of UDPGlcNAc[6] (Kahan, F., personal communication). Neither the UDPMur NAc-tripeptide nor the pentapeptide can replace the requirement of UDPGlcNAc for the irreversible inactivation by phosphonomycin (data not shown). Moreover, the inhibition of PEP:UDPGlcNAc enolpyruvyltransferase by either UDPMurNAc-tripeptide or -pentapeptide was independent of the concentration of UDPGlcNAc, the extent of inhibition being the same when UDPGlcNAc concentrations were varied from 0.85 to 17 mM. Thus it appears that the binding site of this enzyme for the inhibitor is distinct from that for one of the two substrates, UDPGlcNAc.

We compared the inhibition of the activities of PEP:UDPGlcNAc enolpyruvyltransferases from *E. coli* strain KMBL-146 and from *B. cereus* by UDPMurNAc-tri- and -pentapeptides. The results are shown in Table II. Though an accumulation of UDP-*N*-acetylmuramyl pentapeptide can be induced by vancomycin in *B. cereus* but not in *E. coli* strain KMBL-146 no significant difference was observed between the sensitivities of the PEP:UDPGlcNAc enolpyruvyltransferases from *E. coli* and *B. cereus* toward inhibition by UDPMurNAc-tri- and -pentapeptides. Thus the presence or absence of accumulation of peptidoglycan precursors *in vivo* cannot be attributed simply to the feedback control on this first enzyme in peptidoglycan biosynthesis by UDP-*N*-acetylmuramyl peptides.

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TABLE II

INHIBITION OF PEP:UDPGlcNAc ENOLPYRUVYLTRANSFERASES FROM *E. coli* K-12 (STRAIN KMBL-146) AND *B. cereus* BY UDPMurNAc-TRI- AND -PENTAPEPTIDES

Preparation of enzyme extracts from *E. coli* KMBL-146 and from *B. cereus*, $(NH_4)_2SO_4$ fractionation and assay procedure are the same as described in the Materials and Methods and in the legend to Table I

Inhibitor (mM)	*Relative enzyme activity*	
	E. coli	*B. cereus*
None	100	100
UDPMurNAc-L-Ala-D-Glu-*meso*-Dpm		
1 mM	112	108
5 mM	68	64
10 mM	37	27
20 mM	10	11
UDPMurNAc-L-Ala-D-Glu-*meso*-Dpm-D-Ala-D-Ala		
1 mM	82	91
5 mM	70	63
10 mM	42	20
20 mM	21	4

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The Author and Subject index of this volume can be found on p. 559 and p. 562, respectively.