BIOSYNTHESIS OF THE PEPTIDOGLYCAN OF *ESCHERICHIA COLI* K-12

Properties of the in vitro polymerization by transglycosylation

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Received 6 December 1979

1. Introduction

In the biosynthesis of bacterial peptidoglycan polymerization proceeds at the expense of the lipid intermediate *N*-acetylglucosaminyl-*N*-acetylmuramyl-(pentapeptide)-pyrophosphoryl-undecaprenol by the formation of the linear glycan strands (transglycosylation step) and the cross-linking of the peptide subunits (transpeptidation step) [1]. A convenient in vitro assay specific for the transglycosylation step was described in [2]. The in vitro formation of polymerized uncrosslinked peptidoglycan material from the lipid intermediate was catalysed by particulate fractions from *Escherichia coli* in the presence of sodium deoxycholate and penicillin G. Here we report general properties of this in vitro polymerization reaction as well as a more complete investigation of the effect of different antibiotics. The importance of this reaction is stressed by the fact that purified penicillin binding protein 1b alone can catalyse a similar reaction [3,4].

2. Materials and methods

Experiments were carried out with particulate fractions prepared as [2] from cells of *E. coli* K 12 Hfr H. Antibiotics 8036 RP and 11837 RP were kindly provided by Dr L. Ninet (Rhône-Poulenc, Vitry), diumycin and prasinomycin by Dr A. Slusarchyk (Squibb Inst. Medical Res., Princeton, NJ) enduracidin by Dr B. Lugtenberg (Dept. Microbiol., Utrecht), mecillinam by Dr F. Lund (Leo Pharmaceut. Prod., Ballerup) and moenomycin by Dr G. Huber (Farbwerke, Hoescht AG, Frankfurt a/M). Penicillin G was purchased from Calbiochem (Hoechst, Paris).

3. Results and discussion

3.1. *Effect of penicillin on the transglycosylation reaction*

When the in vitro transglycosylation reaction was performed with particulate fractions from *E. coli* K-12 in the absence of any penicillin, the lipid intermediate was rapidly degraded, only low amounts of polymerized material were formed and ~50% of the [^{14}C]alanine of the substrate was released (table 1). In *E. coli* different penicillin-sensitive D-alanine carboxypeptidase activities, which catalyse the release

Table 1
Effect of penicillin G on the in vitro transglycosylation reaction

Penicillin G final conc. (μg/ml)	Labelled reaction products (dpm)		
	Lipid intermediate	Alanine	Peptidoglycan
0	1915	2768	395
1	1180	2465	570
10	1105	1985	1565
100	1565	395	2345
500	1605	380	3040

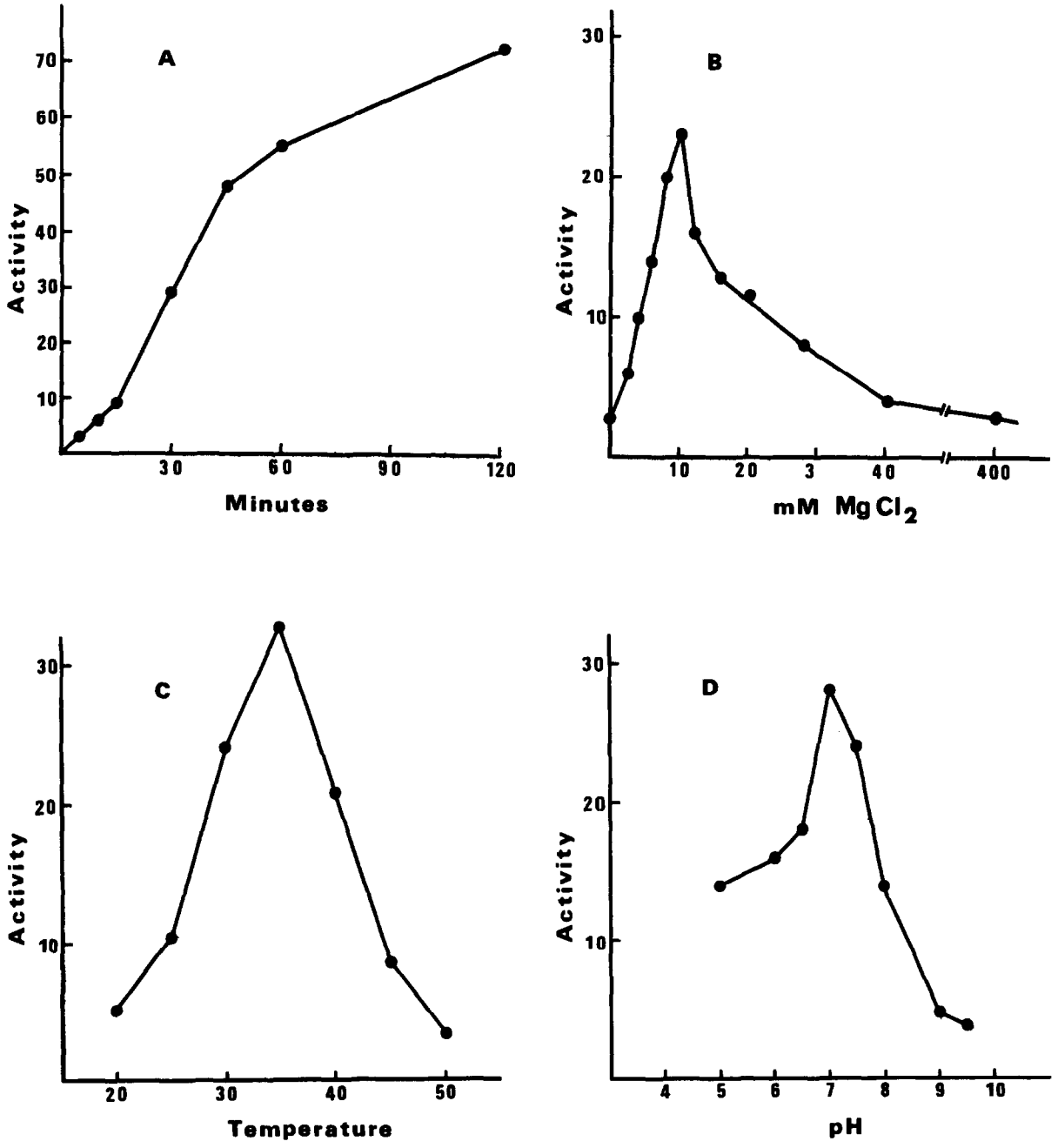

Fig.1. Time course of the in vitro transglycosylation reaction (A) and effect of [Mg^{2+}] (B), temperature (C) and pH (D) on the initial velocity of the reaction. The normal assay for the transglycosylation activity was carried out by incubating at 30° C for 30 min a mixture containing, in 30 μl final vol., 0.2 M Tris–HCl buffer (pH 7.5), 0.01 M $MgCl_2$, 0.1 mM lipid intermediate (8000 dpm), 0.05% penicillin G, 0.1% sodium deoxycholate and particulate fraction (150 μg protein). The reaction products were separated and quantified as in [2]. Activities are expressed as the % of the radioactivity in the polymerized material to that in the polymerized material and in the remaining lipid intermediate. At pH 5–7 Tris–maleate buffers were used whereas at pH 7.5–9.5 Tris–HCl buffers were used.

of the C-terminal D-alanine residue from the uridine-diphospho-*N*-acetylmuramyl-pentapeptide precursor, have been described as membrane-located enzymes [5]. Presumably these D-alanine carboxypeptidase activities catalyse in a similar way the release of the C-terminal [^{14}C]alanine from the lipid intermediate. With the addition of increasing amounts of penicillin the release of alanine decreased whereas the formation of peptidoglycan material was enhanced (table 1). These results showed that the addition of penicillin is necessary for the assay and suggested that the lipid intermediate containing a tetrapeptide subunit is not a suitable substrate for the transglycosylation reaction. Since the transpeptidation step is also sensitive to the effect of penicillin [1] only uncrosslinked peptidoglycan is formed in its presence [2] and the assay is specific for the transglycosylation step.

3.2. *Properties of the in vitro transglycosylation reaction*

After a lag period of a few minutes, the time course of the formation of peptidoglycan (fig.1A) was essentially linear for ~30 min. It is noteworthy that the lipid intermediate is stable upon storage over long periods of time (⩾1 year) at −25°C in chloroform–methanol (1:1). However, its stability under the conditions of the assay (30–40°C) is poor for >1 h when the extent of polymerization is low (absence of sodium deoxycholate or presence of an inhibitor such as moenomycin). The polymerizing activity of particulate fractions is stable for several months at −25°C. Under the conditions of the in vitro transglycosylation assay, the reaction was found to be greatly dependent on [Mg^{2+}]. A sharp optimum at 10 mM was observed (fig.1B). The temperature optimum of the reaction is at about 35°C (fig.1C) and the pH optimum at 7 (fig.1D).

3.3. *Effect of antibiotics*

It was shown that antibiotics 11837 RP and moenomycin had a specific inhibiting effect on the in vitro transglycosylation reaction [2]. Further results were obtained by examining the effect of antibiotics such as 8036 RP, diumycin, enduracidin, prasinomycin (table 2). The ID_{50} values of these compounds were 0.01–0.1 μg/ml, except for enduracidin which was a less efficient inhibitor. These results further stress the fact, substantiated [6], that close analogies exist between these different compounds.

Table 2
Effect of antibiotics on the transglycosylation reaction catalysing the formation of peptidoglycan

Antibiotic	Final conc. (μg/ml)	% inhibition
8036 RP	0.1	100
	0.01	56
11837 RP	0.1	95
	0.01	5
Diumycin	0.1	100
	0.01	51
Enduracidin	1	23
	0.1	0
Moenomycin	0.1	100
	0.01	39
Prasinomycin	0.1	100
	0.01	60
Mecillinam	10	0

As expected from the results in [7] mecillinam had no inhibiting effect on the reaction.

4. Conclusion

The data presented in [3] strongly suggest that penicillin binding protein 1b is responsible for the transglycosylation reaction described here. The properties of the polymerizing activity associated with this specific membrane protein should now be investigated and compared to those reported here for the reaction catalysed by particulate fractions. Undoubtedly, such a comparison would greatly help to further substantiate the identity of the two activities.

Acknowledgements

This investigation was supported by grants from the CNRS (Equipe de recherche no. 15) and the DGRST (grant no. 75-7-0050).

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