EFFECT OF BACITRACIN ON THE BIOSYNTHESIS OF DOLICHOL DERIVATIVES IN CALF PANCREAS MICROSOMES

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1. Introduction

Bacitracin, a cyclic polypeptide antibiotic produced by *Bacillus licheniformis*, has a variety of inhibitory effects in bacterial and animal cells. It blocks the biosynthesis of cell-wall peptidoglycan by preventing the dephosphorylation of undecaprenyl pyrophosphate, thereby limiting the availability of undecaprenyl phosphate, which is essential for muramic acid pentapeptide incorporation into the cell wall [2]. This inhibition is due to the formation of a complex between bacitracin, divalent cation and undecaprenyl pyrophosphate [3]. Bacitracin also inhibits the biosynthesis of squalene and sterols from mevalonate [4] and prevents the formation of ubiquinones [5] by a similar mechanism. Under some conditions, bacitracin induces permeability changes in protoplasts that result from its interaction with undecaprenyl pyrophosphate molecules in the membrane [6]. More recently, it has been shown to inhibit the growth of *Halobacterium salinarium*, a bacterium which contains a glycoprotein as one of its major cell-wall components [7].

We have shown [1,9–11] that calf-pancreas microsomes catalyze the following reactions which are important in the biosynthesis of glycoproteins (for review see [8]):

Dol-P + UDP-GlcNAc → Dol-PP-GlcNAc + UMP

Dol-PP-GlcNAc + UDP-GlcNAc → Dol-PP-$(GlcNAc)_2$ + UDP

Dol-P + GDP-Man → Dol-P-Man + GDP

Dol-P + UDP-Glc → Dol-P-Glc + UDP.

In the present report, we demonstrate that bacitracin inhibits the formation of Dol-PP-GlcNAc, but has relatively little effect on the synthesis of Dol-PP-$(GlcNAc)_2$, Dol-P-Man, and Dol-P-Glc.

Abbreviations: Dol-P, dolichyl phosphate; Dol-PP-GlcNAc, P^1-2-acetamido-2-deoxy-α-D-glucopyranosyl P^2-dolichyl pyrophosphate; Dol-P-Man, dolichyl β-D-mannopyranosyl phosphate; Dol-P-Glc, dolichyl β-D-glucopyranosyl phosphate; Dol-PP-$(GlcNAc)_2$, P^1-di-acetyl-α-chitobiosyl P^2-dolichyl pyrophosphate

Lipid Intermediates of Complex Polysaccharide Biosynthesis, part XIV. (For part XIII, see ref. [1])

2. Materials and methods

2.1. *Materials*

The source of chemicals is described in preceding publications [1,12]. GDP-D-[U-^{14}C]mannose (220–246 mCi/mmol) and UDP-D-[U-^{14}C]glucose (139 mCi/mmol) were obtained from New England Nuclear, Boston, MA, and UDP-*N*-acetyl-D-[U-^{14}C]glucosamine (300 mCi/mmol) from Amersham/Searle Corp., Arlington Heights, IL. Bacitracin was a product of either Sigma Chemical Co., St Louis, MO or the Upjohn Co., Kalamazoo, MI.

2.2. *Preparation of microsomes*

Calf pancreas microsomes were prepared by method 2 as described previously [1].

2.3. *Enzyme assays*

The biosynthesis of Dol-P-Man, Dol-P-Glc, and Dol-PP-GlcNAc was measured as described previously

[1,11,12]. Microsomes were incubated for 30 min at 30°C in total vol. 0.5 ml containing 5–6 mg protein/ml, Tris-maleate buffer (40 mM), $MnCl_2$ (10 mM), 2-mercaptoethanol (10 mM) and either GDP-D-[U-^{14}C] mannose, UDP-D-[U-^{14}C]glucose, or UDP-*N*-acetyl-D-[U-^{14}C]glucosamine at a concentration of 0.05 μCi/ml. The pH of the buffer varied with the nucleotide sugar precursor and with the experiment. In experiments with exogenous dolichyl phosphate, the appropriate amount of dolichyl phosphate solution in chloroform–methanol (2:1) was first added to the tubes, the solvent was evaporated under N_2, and the other constituents were added as just described, including Triton X-100 at a final concentration of 0.1%. For the biosynthesis of Dol-PP-$(GlcNAc)_2$, the medium contained synthetic Dol-PP-GlcNAc (20 μg/ml), sodium taurocholate (0.5%), Tris-maleate buffer, pH 7.3 (40 mM) and UDP-*N*-acetyl-D-[U-^{14}C]glucosamine (0.1 μCi/ml) in total vol. 0.25 ml.

At the end of the incubation, the tubes were cooled to 4°C and 5 vol. chloroform–methanol (2:1, v/v) were added. The suspension was mixed with a Vortex mixer, kept at room temperature for 10–20 min, mixed again and centrifuged. The lower chloroform–methanol extract was removed with a Pasteur pipette and its radioactivity was determined by counting an aliquot in 8 ml Aquasol (New England Nuclear, Boston, MA) with a Packard liquid scintillation counter model 3375. The results presented are averages of duplicate incubations.

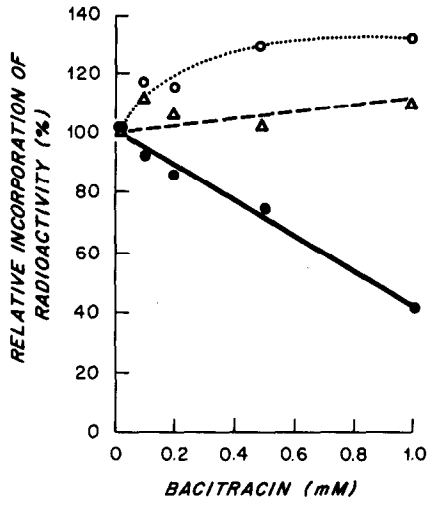

Fig.1. Effect of bacitracin on the formation of dolichol derivatives. The conditions of the experiments are described in Materials and methods. Incubation with: (●———●)UDP-*N*-acetyl-D-[U-^{14}C]glucosamine, at pH 7.3; (△———△)GDP-D-[U-^{14}C]mannose, at pH 6.3; (○———○)UDP-D-[U-^{14}C]glucose, at pH 5.3. The results are expressed as % radioactivity in the absence of bacitracin. These values were 2160 cpm, 13 400 cpm and 3300 cpm, respectively for dolichol derivatives of *N*-acetylglucosamine, mannose and glucose.

3. Results and discussion

Bacitracin greatly inhibited the incorporation of radioactivity from UDP-*N*-acetyl-D-[^{14}C]glucosamine into the chloroform–methanol extract (fig.1). Under these conditions of incubation, 80–85% of the radioactivity was recovered in Dol-PP-GlcNAc and the rest in Dol-PP-$(GlcNAc)_2$ when the chloroform–methanol extract was subjected to thin-layer chromatography on silica gel G in chloroform–methanol–water (60:35:6, by vol.). Bacitracin inhibited the labeling of both glycolipids to the same extent. However, when the formation of Dol-PP-$(GlcNAc)_2$ from synthetic Dol-PP-GlcNAc and UDP-*N*-acetyl-D-[^{14}C]glucosamine was measured, it was found that bacitracin (1 mM) decreased its formation by only about 10%. These results show that bacitracin inhibits the synthesis of Dol-PP-GlcNAc and has little effect on the formation of Dol-PP-$(GlcNAc)_2$ from Dol-PP-GlcNAc. This inhibition was concentration dependent and observed with bacitracin obtained from two different sources. In contrast, bacitracin did not inhibit the formation of Dol-P-Man and Dol-P-Glc, the glycolipids found in the chloroform–methanol extract after incubation with GDP-[^{14}C]mannose and UDP-[^{14}C]glucose respectively (fig.1). In fact, some stimulation of Dol-P-Glc and of Dol-P-Man synthesis was observed. It is of interest to note that Sentandreu et al. [13] also reported that bacitracin enhanced Dol-P-Man synthesis in yeast.

It has been shown that the formation of a complex between bacitracin, cation, and C_{55}-isoprenyl pyrophosphate is pH dependent with an optimum pH between 7.0 and 7.5 [14]. Since the reactions described in fig.1 were performed at the optimum pH for each enzyme assay, it was important to determine whether the differences observed were related to the pH of the incubation. When the experiments were repeated with all incubations performed at pH 7 at the

Table 1
Effect of bacitracin on Dol-PP-GlcNAc synthesis in the presence and absence of exogenous dolichyl phosphate

Bacitracin (mM)	Radioactivity in chloroform–methanol extract (cpm)	
	Without Dol-P	With Dol-P
0	1070	1190[a]
0.5	560	840[a]
1.0	470	690[a]
0	2050	2950[b]
1.0	1270	1970[b]

[a]Dol-P concentration: 40 μg/ml
[b]Dol-P concentration: 160 μg/ml

Incubation was performed at pH 7.3 with UDP-*N*-acetyl-D-[^{14}C]glucosamine as described under Materials and methods

same cation concentration, the results were similar in that bacitracin inhibited the formation of Dol-PP-GlcNAc without affecting that of Dol-P-Man and Dol-P-Glc.

If the inhibitory action of bacitracin were due to complex formation with dolichyl pyrophosphate, thereby decreasing the availability of dolichyl phosphate, it should be possible to reverse the effect by the addition of exogenous dolichyl phosphate. It was found, however, that the inhibitory action of bacitracin on Dol-PP-GlcNAc synthesis was still very significant in the presence of a relatively large concentration of exogenous dolichyl phosphate (table 1). These results suggest that bacitracin has an effect on the enzyme involved in Dol-PP-GlcNAc biosynthesis rather than an indirect effect due to depletion of lipid substrate, such as that observed in bacterial cell-wall biosynthesis [3]. The effect of bacitracin may be due to membrane disruption [15] or may be a direct effect on the enzyme in pancreas microsomes. Chen and Lennarz [16] found that, in hen oviduct membranes, incubation with UDP-*N*-acetyl-D-[^{14}C]glucosamine in the presence of bacitracin resulted in the accumulation of labeled *N*-acetylglucosamine-containing lipids, but the mechanism of this effect was not clarified. It is not known whether bacitracin interferes with glycoprotein biosynthesis in intact cells at the concentrations used in the present experiments, but it seems possible that the inhibitory action of bacitracin reported here is related to the toxicity of this drug in vivo [17] since the formation of Dol-PP-GlcNac is an important step in the biosynthesis of glycoproteins containing an *N*-glycosylic linkage between asparagine and *N*-acetylglucosamine [8].

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