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Crystallization of the Soluble Lytic Transglycosylase from *Escherichia coli* K12

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Lytic transglycosylases degrade the murein polymer of the bacterial cell wall to 1,6-anhydromuropeptides. These enzymes are of significant medical interest, not only because they are ideal targets for the development of new classes of antibiotics, but also because the low molecular weight products of their catalytic action can cause diverse biological activities in humans, which can be either beneficial or toxic. A soluble lytic transglycosylase was purified from an overproducing *Escherichia coli* strain and X-ray quality crystals were obtained at room temperature from hanging drops by vapor diffusion against 20 to 25% $(NH_4)_2SO_4$, in 100 mm-sodium acetate buffer, pH 5·0. The crystals diffract in the X-ray beam to 2·8 Å resolution. Their space group is $P2_12_12_1$ with cell dimensions $a = 81$ Å, $b = 88$ Å and $c = 135$ Å. Assuming one monomer (M_r 70,362) per asymmetric unit, the solvent content of these crystals is 63%.

The cell wall of most bacteria derives its mechanical stability from murein, a structurally unique biopolymer that surrounds the whole bacterial cell as a single macromolecule. This polymer is composed of glycan strands of variable length that are crosslinked by short peptide bridges, thus forming a network-like structure. The integrity of the murein polymer is of vital importance to the bacteria: uncontrolled cleavage of bonds will finally cause lysis of the cells (Schwarz *et al.*, 1969). Nevertheless, the cell wall polymer must undergo partial hydrolysis and resynthesis during bacterial growth and cell division. For this purpose the bacterium possesses a strictly regulated set of enzymes, such as murein hydrolases and synthetases. Because murein is unique to bacteria, these enzymes are ideal targets for selective inhibition, and thus for the development of antibiotics (for a review, see Höltje & Schwarz, 1985). Penicillin and other beta-lactam compounds inhibit several of these enzymes, but others, such as the transglycosylases are not affected by beta-lactam antibiotics.

Among the murein-metabolizing enzymes our interest focuses on the lytic transglycosylases, which are able to degrade the murein polymer completely to form soluble low molecular weight products. Two lytic transglycosylases have been found in *Escherichia coli*: a soluble enzyme of molecular weight 70,362 that occurs in the periplasm (Engel & Keck, unpublished results), and a membrane-bound enzyme with a molecular weight of 35,000 (Keck *et al.*, 1985). Both transglycosylases catalyze the cleavage of the β-1,4-glycosidic bond between *N*-acetylmuramic acid and *N*-acetylglucosamine. In this respect both transglycosylases have lysozyme activity. However, in addition these enzymes perform, after the cleavage, an intramolecular muramyl transferase reaction, thereby creating muropeptides with a 1,6-anhydromuramic acid (Taylor *et al.*, 1975; Höltje *et al.*, 1975). Quite unexpectedly, it was found that the sleep-inducing factor, which was isolated from human urine
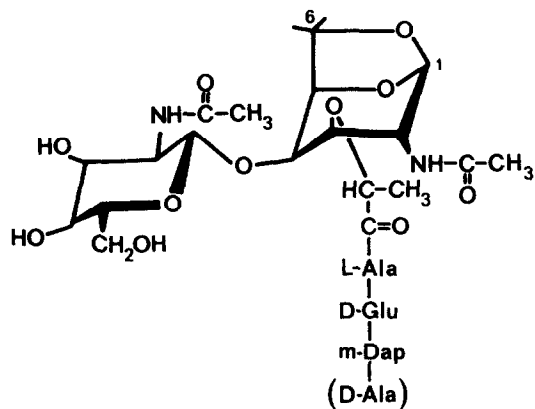
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Figure 1. Structure of the 1,6-anhydro-disaccharide-tetrapeptide released by the lytic transglycosylase from murein polymer.

(Martin et al., 1984) and that was shown to promote slow-wave sleep when injected in picomolar amounts cerebro-intraventricularly into rabbits (Krueger et al., 1984), is structurally identical to the monomeric product (N-acetylglucosaminyl-1,6-anhydro - N - acetylmuramyl - L - alanyl - D - isoglutaminyl-m-diaminopimelyl-D-alanine; Fig. 1) of these enzymes (Höltje et al., 1975). The identical compound is shown to elicit cytotoxic effects on ciliated epithelial cells when released from human pathogenic microorganisms like Bordetella pertussis and Neisseria gonorrhoeae (Cookson et al., 1989; Sinha & Rosenthal, 1980).

Here we describe the crystallization of the soluble transglycosylase from E. coli. The structural gene of this enzyme has been cloned, and for ease of purification an overexpressing E. coli strain has been established (Betzner & Keck, 1989). The enzyme was purified by combination and modification of the previously described protocols (Höltje et al., 1975; Kusser & Schwarz, 1980). The purification to homogeneity of 100 mg transglycosylase was achieved by the following successive steps. The crude extract from 280 g wet weight of E. coli cells was dialysed against Tris-maleate/NaOH buffer (10 mM, pH 6·8) and loaded onto a CM-Sepharose CL-6B column. The transglycosylase was eluted with a linear gradient, at 0·3 M-NaCl. After dialysis against the same Tris-maleate/NaOH buffer the enzyme pool obtained was chromatographed over a Blue Sepharose CL-6B column from which it was eluted at 0·2 M, with a linear NaCl gradient. The final purification and concentration of the transglycosylase to a concentration of 10 mg/ml was carried out on a hydroxylapatite column in potassium phosphate buffer (10 mM, pH 6·8). The peak fraction eluted at 0·1 M on a potassium phosphate gradient.

The protein thus purified was submitted to crystallization trials by vapor diffusion in hanging drops. Several precipitating agents were tried: ammonium sulfate, polyethylene glycol 6000, sodium chloride and sodium citrate in the pH range from 3·5 to 8·0. After a few weeks small crystals

were grown at room temperature from 15 to 30% ammonium sulfate in 0·1 M-sodium acetate buffer, pH 4·5 to 5·0. The average dimensions of the crystals were 0·2 mm × 0·05 mm × 0·05 mm. The X-ray quality of these crystals was no better than 5 Å resolution (1 Å = 0·1 nm). Seeding was used to grow larger crystals. A small single crystal was washed in 20% ammonium sulfate in 0·1 M-sodium acetate buffer, pH 5·0. The seed, together with 3 μl of the washing solution was transferred to a 6 μl drop containing 3 mg transglycosylase/ml and 15% ammonium sulfate in buffer, pH 5·0. These drops were equilibrated against 1 ml of a solution containing 20% ammonium sulfate in buffer, pH 5·0. After two to three days the concentration of the ammonium sulfate in the well was gradually increased (within one week) to 25% ammonium sulfate. By this technique crystals grow with dimensions of 0·6 mm × 0·2 mm × 0·1 mm. For X-ray studies the crystals are transferred to a stabilizing solution of 30% ammonium sulfate in sodium acetate buffer, pH 5·0.

Precession photographs indicate that the crystals are orthorhombic, with space group $P2_12_12_1$ and unit cell parameters $a = 81$ Å, $b = 88$ Å and $c = 135$ Å, which corresponds to a unit cell volume of 962,280 Å^3. Assuming that we have one molecule (70,000 Da) per asymmetric unit the volume per unit mass, V_M (Matthews, 1968) is 3·4 Å^3/dalton. This is within the range 1·6 to 3·6 Å^3/dalton that was found to be typical for protein crystals. The solvent content is then 63%. The crystals diffract to about 2·8 Å resolution. A native dataset has been collected by oscillation photography on the image plate at the EMBL outstation at the DESY synchrotron in Hamburg. A search for heavy-atom derivatives is under way.

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