| ESRF | **Experiment title:** **Structural Studies on glycosyltransferase SpsA from *Bacillus subtilis*** | **Experiment number:** Ls1070/ls1071 |
|---|---|---|
| **Beamline:** ID14-4 | **Date of experiment:** from: 15/12/98 to: 17/12/99 | **Date of report:** 27/08/99 |
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Report:

Nucleotide-diphospho-sugar transferases are a ubiquitous group of enzymes which catalyse the formation of di, oligo and polysaccharides, lipopolysaccharides and the glycosylation of proteins. This is performed by the transfer of a sugar moiety from an activated nucleotide-diphosphate (NDP) sugar donor. Whilst, in terms of quantity at least, the NDP-sugar transferase are the most important enzymes on Earth, little is known about their structure or catalytic mechanism. We have been using the ESRF to study nucleotide and inhibitor complexes of an NDP-sugar transferase: SpsA from *Bacillus subtilis*.

SpsA is an NDP-sugar transferase involved in spore-coat synthesis in sporulating *Bacilli*. It has been expressed in an *E. coli* system and crystals prepared. The structure was originally solved using data collected at the Daresbury SRS [1]. The 256 amino-acids of SpsA fold to reveal forms 2 domains, Figure 1 [2]. The N-terminal 100 amino acids form a nucleotide-binding domain consisting of a 4 stranded parallel β-sheet flanked on either side by 2 α-helices. The C-terminal domain, which may be the binding site for the acceptor species, is of mixed topology.

Using data collected at the ESRF, beamline ID14-4, we have studied the substrate specificity of SpsA. Data for complexes with UDP, Mn-UDP, $Mn^{2+}$ alone and $Mg^{2+}$-TDP were collected. In addition, data were collected with the putative inhibitor Nikkomycin, unfortunately this did not bind in-crystal. In the Mg-UDP, Mn-UDP and Mg-TDP complexes the nucleotide binds, as expected to the nucleotide-binding domain in a deep cleft. The ribose adopts a C2'-*endo* conformation. The base moiety binds both through aromatic stacking with Tyr 11 and through a number of hydrogen-bonding interactions. Asp 39, an invariant residue at the end of strand β-2, hydrogen-bonds with the N3 of the uracil base. Asp 39 is one of two invariant aspartates which are signature motifs of "family 2" of the glycosyltransferases (the other being Asp 99) and whose function has been long speculated upon, especially in the related cellulose synthase system. In the Mn-UDP complex, a single $Mn^{2+}$ ion is found located between the two phosphates, which in turn interacts with the protein *via* Asp 99. This invariant residue lies on β-strand 4 where it most likely assists leaving-group departure through coordination of the divalent manganese ion, Figure 2.

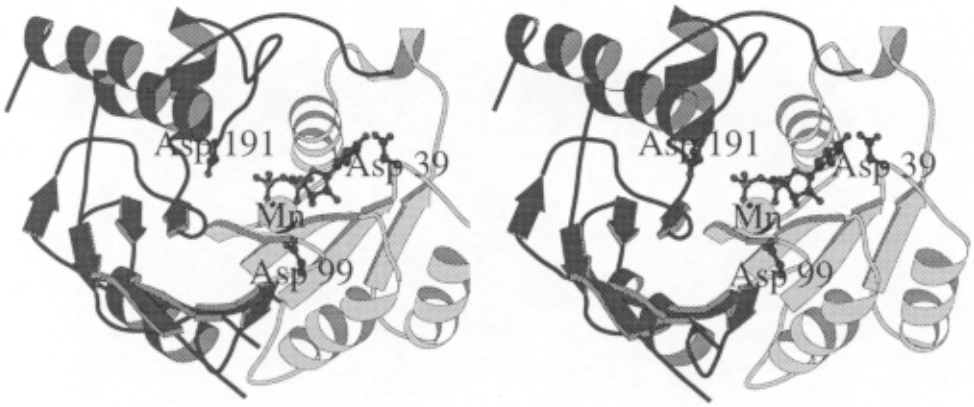


*Figure 1.* The topology of SpsA. This figure was drawn with the MOLSCRIPT program [Kraulis, P.J. (1991) *J. Appl. Cryst.* 24, 946 – 950].

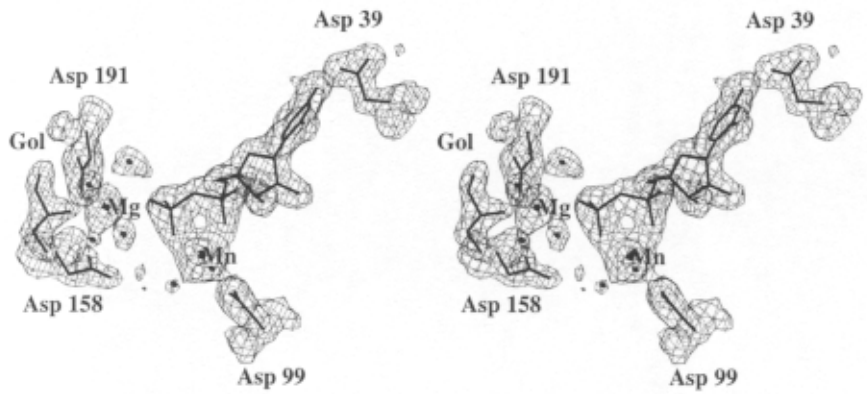


*Figure 2.* Observed electron density for Mn-UDP binding to glycosyltransferase SpsA from *Bacillus subtilis*, using data collected from ID14-4.

The importance of glycosyltransferases, particularly the nucleotide-diphospho-sugar dependent enzymes, serves only to emphasise our lack of catalytic and structural knowledge on these systems. Data collected at the ESRF has permitted an analysis of nucleotide specificity for the first nucleotide-diphospho-sugar transferase from glycosyltransferase family 2. Other family 2 members, including those of well-defined substrate specificity, display a much higher complexity than SpsA, often including multiple domains and membrane-spanning regions. The structure of SpsA provides an initial glimpse into the active centre of family 2 glycosyltransferases and will act as the precursor to the study of these other more challenging systems.

## References

[1]. Charnock, S.J. & Davies, G.J. Cloning, crystallisation and preliminary X-ray analysis of a nucleotide-diphospho-sugar transferase, SpsA, from *Bacillus subtilis*. *Acta Cryst* **D 55**, 677-678 (1999).

[2]. Charnock, S.J & Davies, G.J. The structure of the nucleotide-diphospho-sugar transferase, SpsA from *Bacillus subtilis,* in native and nucleotide-complexed forms. *Biochemistry* **38**, 6380-6385 (1999).