ESRF	**Experiment title:** The rhamnose pathway, a target for therapeutic intervention	**Experiment number**: WT-12
Beamline: ID14EH2	**Date of experiment**: from: 02.11.00 to: 03.11.00	**Date of report**: 04.12.00
Shifts: 3	**Local contact(s)**: Stéphanie Monaco	*Received at ESRF:*

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Report:

L-rhamnose is a key residue of the cell wall of many pathogenic bacteria. It is generated from glucose-1-phosphate in a synthesis involving four enzymes (termed RmlA to RmlD), yielding dTDP-L-rhamnose as the precursor for cell wall generation. As L-rhamnose is not found in humans and as the enzymes of the rhamnose pathway show a high degree of conservation amongst microorganisms the pathway is an attractive target for the development of novel antibiotics. RmlC catalyses the epimerisation at positions 3 and 5 of the glucose ring, which leads to a subsequent ring flip. Thus, RmlC is the key enzyme for the stereochemistry of L-rhamnose biosynthesis. The day on ID14EH2 was spent to collect complex data for the first three enzymes in the pathway as well as to measure a data set for an RmlB protein previously unknown, namely RmlB from *Streptococcus suis*. The structure of RmlA (glucose-1-phosphate thymidylyltransferase; E.C. 2.7.7.24) from *Pseudomonas aeruginosa* has recently been accepted for publication[1]. The protein catalyses the transfer of a 2'-deoxythymidine monophosphate group to glucose-1-phosphate, generating dTDP-D-glucose. Our previous work allowed to derive an almost complete insight into the catalytic mechanism of this enzyme, yet an uncertainty concerning the Mg^{2+}-dependency of the reaction remained as cocrystallisation experiments with metal ions had not been successful previously. We collected a dTDP-D-glucose complex of RmlA in which 0.2 M of the precipitant's 0.5 M Li_2SO_4 had been replaced by $MgSO_4$. Density maps of this complex are currently being investigated. A paper describing the structure of RmlB (dTDP-D-glucose 4,6-dehydratase; E.C. 4.2.1.46) from *Salmonella enterica*] has been submitted lately. Whilst working on the ESRF-beamline ID14EH2 we were able to collect a native data set of RmlB from *Streptococcus suis*. This protein has a sequence identity of ca. 38 % to the known structure. Crystallisation of the enzyme has been especially difficult as two promising looking crystal forms have not given any diffraction at all and a third condition resulted in needle-shaped crystals with small dimensions, making the use of a high intensity synchrotron beamline especially desirable. A straightforward molecular replacement allowed us to derive initial phases for the data collected at the ESRF. The structure of *S. suis* RmlB is currently being refined.

RmlC (6-deoxy-D-xylo-4-hexulose 3,5-epimerase; E.C. 5.1.3.13), the third enzyme in the rhamnose pathway, is a novel epimerase that has been published earlier this year. In an experiment carried out on beamline BM14

(see other report) we solved using SAD data the *S. Suis* RmlC dimer in the $P2_1$ asymmetric unit and to generate a rough Ca trace of sufficient quality to determine the cross-crystal averaging operators for two high resolution data collected on ID14EH2. At a maximum resolution of 1.6 ? automated protein structure refinement with following manual adjustments to the model was a question of hours. A detail of the final electron density of *S. suis* RmlC can be seen in Figure 1. The configuration of the active center of *S. suis* RmlC together with the four complex data for *P. aeruginosa* and *S. Suis* RmlC collected on ID14EH2 will provide us with further insights into the interesting stereochemistry of RmlC catalysis.

Figure 1

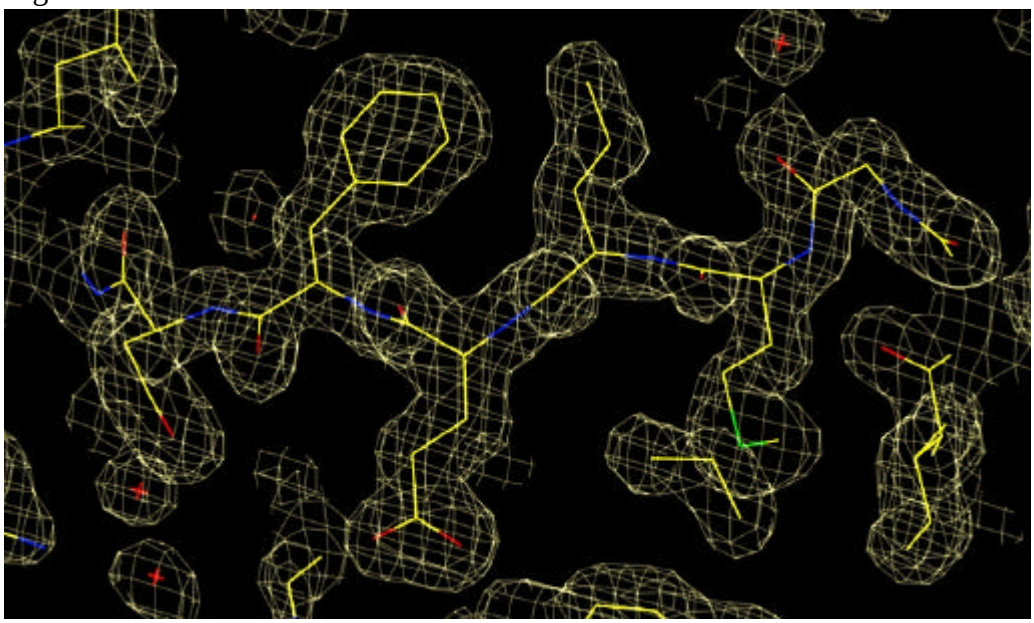

Table I

Protein	***P. aeruginosa* RmlA dTDP-D-glucose, $MgSO_4$**[a]	***S. suis* RmlB**	***C. methanomorphum* methyl aspartase**
Resolution (Highest Shell, Å)	55.0 – 1.52 (1.60 – 1.52)	52.0 – 2.80 (2.95 – 2.80)	50.6 – 2.0 (2.11 – 2.0)
Space group	P1	$P2_12_12_1$	$P2_12_12$ or $P222_1$
Cell constants (Å; º)	71.5, 73.5, 134.2; 89.9, 80.7, 81.2	61.8, 96.2, 184.5; 90.0, 90.0, 90.0	67.2, 109.9, 110.3; 90.0, 90.0, 90.0
Total measurements	477833	148967	226095
Unique reflections	227547	26272	53841
Average redundancy	2.0 (1.7)	5.7 (5.2)	4.2 (4.0)
I/s	12.1 (4.5)	3.5 (1.3)	7.0 (3.4)
Completeness (%)	58.3 (5.0)	94.4 (91.3)	96.8 (98.1)
$\mathbf{R_{merge}}$	3.3 (15.0)	17.3 (47.1)	7.4 (19.9)

All data has been collected at 0.933 ?. Processing was done with MOSFLM/SCALA.
[a] Data is complete to ca. 1.8 ? .

Table II

Protein	***P. aeruginosa* RmlC, product complex**	***S. suis* RmlC, product complex**	***S. suis* RmlC, dTDP-D-glucose complex**
Resolution (Highest Shell, Å)	48.2 – 1.70 (1.79 – 1.70)	48.2 – 1.80 (1.90 – 1.80)	44.3 – 1.60 (1.69 – 1.60)
Space group	$P2_12_12$	$P2_1$	$P2_1$
Cell constants (Å; º)	64.4, 146.2, 44.9; 90.0, 90.0, 90.0	39.7, 70.5, 67.7; 90.0, 103.9, 90.0	50.6, 47.9, 72.4; 90.0, 99.0, 90.0
Total measurements	245237	124014	153570
Unique reflections	40026	33657	43352
Average redundancy	6.1 (4.7)	3.7 (3.6)	3.5 (2.7)
I/s	5.6 (1.8)	5.4 (1.5)	5.2 (1.8)
Completeness (%)	84.3 (44.1)	99.9 (99.4)	95.7 (79.8)
$\mathbf{R_{merge}}$	8.6 (38.4)	7.6 (37.8)	8.3 (29.2)