4-Hydroxybutyryl-CoA Dehydratase from *Clostridium aminobutyricum*: Preliminary Crystallographic Studies

B.M. Martins, S. Macedo-Ribeiro, I. Çinkaya[1], W. Buckel[1] and A. Messerschmidt*

Strukturforschung, Max-Planck-Institut für Biochemie, Am Klopferspitz 18-a, D-82152 Martinsried, Germany

[1]Laboratorium für Mikrobiologie, Philipps-Universität, Karl-von-Frisch-Str., D-35032 Marburg, Germany

4-Aminobutyrate generated by the α-decarboxylation of glutamate can be use by anaerobic bacteria for energy conservation via an electrochemical proton gradient producing ammonia, acetate and butyrate. In the brain of vertebrates, 4-aminobutyrate acts as an inhibitory neurotransmitter, also known as γ-aminobutyrate or GABA. The strict anaerobic bacterium *Clostridium aminobutyricum* ferments 4-aminobutyrate via 4-hydroxybutyrate to acetate and butyrate, whereby 0.5 ATP/4-aminobutyrate is formed. The mechanistically most interesting reaction of the pathway in *C. aminobutyricum* is the reversible dehydration of 4-hydroxybutyryl-CoA to crotonyl-CoA since one of the two non-activated hydrogens at the β-carbon has to be removed as proton [1]. This key step is catalysed by the oxygen-sensitive enzyme 4-hydroxybutyryl-CoA dehydratase, a homotetramer (4 x 56 kDa) containing one FAD moiety and one [4Fe-4S] cluster per monomer. The catalytic mechanism most probably involves a transient deprotonation and one-electron transfer steps via a ketyl radical anion as intermediate.

The enzyme, purified under strictly anoxic conditions [2] has been crystallised in the presence of crotonyl-CoA and reducing agent under strictly anoxic conditions. Parallelepiped and prism-like crystals appear after 2-4 weeks depending in the type and concentration of the precipitant. The presence of iron-sulphur clusters in the protein permits the use of the multiple-wavelength anomalous dispersion (MAD) method at the absorption edge for iron to determined its three-dimensional structure. Thus, several crystals were shock frozen for MAD measurements at the beam line BW& (DESY, Germany).

Albeit showing a diffraction power up to 3.5 Å, all tested crystals displayed high mosaicity along with anisotropy in the direction of the c-axis. A partial data set was collected at 3.5 Å using a rotating angle of 0.1 °. Preliminary data collection from an in-house x-ray facility (MAR IP coupled to a Rigaku rotating anode x-ray generator) indicated that the crystals belong to the monoclinic crystallographic system. However, the unit cell parameters (a=189.8 Å, b=67.2 Å, c=247-251 Å, α=91.1°, β=107.9° and γ=90.2°) using synchrotron radiation indicates that the space group is most probably P1. Thus the diffraction quality of these crystals was not sufficient for MAD data collection. Work is on going to optimise both the harvesting and cryoprotectant solution in order to try to reduce the mosaicity.

**Present address: CNC, Faculdade de Medicina, Universidade de Coimbra, 3049 Coimbra codex, Portugal*

References

[1] W. Buckel, FEBS Lett, 389, 20 (1996)

[2] A. Gerhard, I. Çinkaya, D. Linder, G. Huisman and W. Buckel, Arch. Microbiol., 174, 189 (2000)