



Contribution ID: 69

Type: **Poster contribution**

## Structural changes during the enzymic action of isopropylmalate dehydrogenase

Thursday, 5 July 2012 10:30 (1h 30m)

IPMDH is essential for leucine biosynthesis in bacteria and plants catalysing the oxidation of 3-isopropylmalate (IPM) to 2-oxo-isocaproate by NAD in the presence of  $Mn^{2+}$ . Single crystal microspectrometry has shown that in crystals of  $IPMDH \cdot MnIPM$  and of  $IPMDH \cdot MnNAD$  the diffusion of the other substrate (respectively NAD or IPM) causes the appearance of the NADH band. The reaction might be limited by diffusion of the substrate or by lattice forces. An active site mutant (K185A) has been produced that exhibits 0.06 % catalytic activity of the native enzyme. The possibility of obtaining a crystal of the mutant enzyme containing both bound substrates before occurring the reaction has been exploited, but resulted in a crystal with the bound reaction products. This structure (2.2 Å,  $R_{free}=23\%$ ) shows an enzyme conformation similar to the  $IPMDH \cdot MnIPM \cdot NADH$  complex, and possibly to the active  $IPMDH \cdot MnIPM \cdot NAD^+$  complex.

We thus plan to grow crystals of the above reported complexes of the mutant enzyme in order to be able to follow the enzymic reaction upon diffusion of the omitted  $NAD^+$  or  $Mn^*IPM$  into them. Simultaneous combination of X-ray diffraction and in situ spectroscopy is expected to reveal the structural changes during catalysis by IPMDH.

**Primary author:** Dr PALLO, Anna (Hungarian Academy of Sciences, Budapest, Hungary)

**Co-authors:** Prof. MERLI, Angelo (University of Parma, Parma, Italy); Dr WEISS, Manfred (Helmholtz-Zentrum Berlin, Berlin, Germany); Prof. VAS, Mária (Hungarian Academy of Sciences, Budapest, Hungary); Dr GRÁCZER, Éva (Hungarian Academy of Sciences, Budapest, Hungary)

**Presenter:** Dr PALLO, Anna (Hungarian Academy of Sciences, Budapest, Hungary)

**Session Classification:** Poster Session

**Track Classification:** Biological / Pharmaceutical Research