ESRF	**Experiment title:** Protein function in the light of conformational changes caused by Ca^{2+}	**Experiment number:** LS 1756
Beamline: ID 14-1	**Date of experiment**: from: 06 / 09 / 2000 to: 07 / 09 / 2000	**Date of report**: 01 / 03 / 2002
Shifts: 3	**Local contact(s):** H. Belrhali	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

V. Harmat*[1], O. Barabas*[1], G. Bunkoczi*[1], J. Debreczeni*[1], A. Gellert*[1], J. Kardos*[2]

1 Lorand Eotvos University, Department of Theoretical Chemistry, Budapest
2 Institute of Enzymology, Hungarian Academy of Sciences

Report:

Aim: We intend to determine the crystal structures of Ca^{2+} complexes of some model proteins in order to examine functional and structural aspects of the biological role of the essential metal ion Ca^{2+}.
All data collections were carried out under cryogenic conditions at beamline ID 14-1.

Physarum myosin II regulatory domain - Ca^{2+} complex. A native dataset (unit cell: a=55.573 Å, b=70.596 Å, c=98.140 Å, $P2_12_12_1$, resolution 1.8 Å, R_{merge}=4.4%) and three derivative (Sm, Ho, Yb) datasets were collected. Phase problem was solved with SIRAS using the Yb derivative (resolution 2.3 Å). (MIRAS did not give better results.)
Problems during model building and refinement: maps at the C terminal domain of the regulatory light chain are of poor quality and B-factors are high for this region. Attempts to improve that region of the model were unsuccessful (arp/warp, CNS, refmac, Shelx tried). Currently R and R_{free} are 0.29 and 0.34, respectively. The disorder may be caused by the His-tag of a symmetry equivalent molecule. Data collection from crystals of His-tag free protein was decided.
Publication: Farkas, L., Debreczeni, J., Harmat, V., Kohama, K., Nakamura, A. and Nyitray, L. (2002): Structure of Ca^{2+}-bound regulatory domain of Physarum myosin II at 1.8 Å resolution: Ca^{2+} is bound to a conventional EF-hand that is in a closed lobe conformation. J. Muscle Res. Cell Motility., in press.

Calmodulin-antagonist complexes. Unit cell of calmodulin-vinblastine crystals is a=b=40.91 Å, c= 341.70 Å, space group $P6_122$ (space group may be $C222_1$ with three fold

non-crystallographic symmetry, a=40.91 Å b=70.87 Å c=341.70 Å). The long cell axis forced us to make a compromise concerning the resolution of the dataset. For acceptable resolution limits overlapping of certain amount of reflections could not be eliminated. We collected a 2.2 Å and a 3.5 Å resolution data sets from one crystal. R_{merge} is 0.112 for the two datasets merged. Phase problem was solved with molecular replacement using data collected previously in our home laboratory. Attempts are being made to improve the quality of refinement (R and R_{free} are 0.35 and 0.40, respectively at the moment).
Crystals of the other calmodulin-antagonist complex (arylalkylamine type designed antagonist) diffracted to 2.6 Å that was not enough for seeing the ligand binding in details (unit cell dimensions a=b=40.53 Å, c=342.34 Å, space group $P6_122$, R_{merge}=0.049).

Chymotrypsin A 1.3 Å resolution dataset was collected from a crystal of alpha-chymotrypsin (a=48.42 Å, b=66.63 Å, c=65.51 Å, β=102.2°, space group $P2_1$, R_{merge}=0.041). Though the structure does not contain bound Ca^{2+} it can be a good reference molecule for studying the conformational effect of Ca^{2+} binding. Refinement is in progress.