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Names and affiliations of applicants (*indicates experimentalists):
* Dr. Yuichiro Makda, IIAR, Panasonic, Seika, Kyoto, Japan 619-02
* Dr. Tomoyoshi Kobayashi, IIAR, Panasonic, Seika, Kyoto, Japan 619-02
* Dr. Soichi Wakatsuki, ESRF, Grenoble, France
* Dr. Min Yao, ESRF, Grenoble, France
Dr. Dmitry G Vassylyev IIAR, Panasonic, Seika, Kyoto, Japan 619-02

Report:

The crystals of tropomyosin (Tm) belong to space group $P4_12_12$ with typical unit cell dimensions: $a=b=107$Å, $c=505$Å. From the very beginning these crystals had two major unique features distinct from other protein crystals; (1) dramatically anisotropic diffraction patterns, which extended up to 3A along c axis and to only 6.6Å along a(b) axis at synchrotron sources; (2) very small signal-to-noise ratios for the reflections between 10 and 5Å along c axis induced by the specific fold of Tm molecules which consist of two long (400Å) coiled-coil α-helices. It was extremely difficult to undertake the X-ray experiments of these crystals using a laboratory source, since they gave very weak diffraction patterns extended only to 4A along c-axis with poor completeness and signal-to-noise ratios even with very long exposure time (3 hours/l degree of rotation). In fact, the experiments at the ESRF in March-July, 1996 were the first trials to obtain reasonable native data, to search for really isomorphous derivatives and also to fix the possible problems in data collection and processing related to these unique features of Tm crystals.

Results.

1 . Two complete data sets have been collected for each of native and two heavy atom derivatives (Pt and Hg) with resolution around 3.5A along c-axis. The total completeness of the data sets is about 45% and typical R-merge about 9%.
2. Self rotation function calculated from the native data revealed non-crystallographic symmetry of two Tm molecules in the asymmetric unit with 45" rotation around c-axis of the crystal.
3. The heavy atom derivatives seem to be rather isomorphous and reasonable heavy atom sites have been found from difference Patterson maps and have been confirmed by analysis of the difference cross-Fourier ED maps.
4. Using MIR phases from these two derivatives a Tm electron density map has been calculated at 4Å resolution, which clearly showed long portions of α-helices extended along c-axis of the crystals. However the map is still not interpretable due to large breaks within the α-helices. The phase improvement techniques like solvent flattening and NCS averaging were not applicable because of these large breaks in the protein electron density and the strongly elongated shape of Tm molecule which did not allow us to build a reasonable molecular envelope.

5. Major problems concerning to crystals, data collection and data processing have been identified:

- The unit cell parameters were not well reproducible even for different native crystals especially along a(b)-axis which means that a success in solving the Tm structure would depend on sheer luck.of finding crystals with the similar cell dimensions in a(b) axis.
- The data collection at room temperature is not available as unfrozen Tm crystals are easily damaged by X-rays and also do not diffract to an atomic resolution (3A).
- The mosaicity of the shock frozen crystals (plunged in the liquid propane before data collection) is typically extremely large, around 1.5-2.5° which dramatically decrease the data quality.
- The oscillation range of 3" used during data collection was too large which strongly influenced on the resulting signal-to-noise ratios of diffraction data and therefore on the final quality of the data sets. An oscillation range of 0.5" gives much better results.
- The Tm diffraction images can not be processed in an usual way when all the predicted spots are integrated by Denzo. Instead a manually generated mask should be used to remove some parts of the dtector active area which do not have reasonable diffraction spots due to the anisotropy of the crystals.

Conclusion.

The efforts should be made to improve the crystal quality and reproducibility of unit cell parameters, and as a consequence the quality and completeness of diffraction data of Tm. Other heavy atom derivatives should be searched in order to improve the MIR phases. After all these experiments, it has now become possible for us to perform proper preliminary X-ray experiments with Tm crystals (derivative search, etc.) using the laboratory X-ray source.

Progress after the ID2 beamtime.

After ESRF experiments with Tm crystals in March-July, 1996, we concentrated our efforts on solving the problems mentioned above. We now found that the majority of these problems (lattice reproducibility and high mosaicity of the shock-frozen crystals) can be solved by partial dehydration of the Tm crystals at room temperature followed by slow flash freezing of the crystals within 4-5 seconds to -150 °C. During this procedure the lattice dimensions along a(b)-axis decrease to a reproducible value of 95Å which is about 10Å shorter than before, the mosaicity drops to a reasonable values of around 0.4-0.7" and the overall signal-to-noise ratio of diffraction pattern is substantially increased. These results, in turn, have given us an opportunity to make more reliable preliminary X-ray experiments with Tm crystals on our rotation anode and a search for new heavy atom derivatives is now well in progress.

Publication as a result of experiments:

A.Miegel, K.Sano, K.Yamamoto, K.Maeda, Y.Maéda, H.Taniguchi, M.Yao & S.Wakatsuki (1996) Production and crystallization of lobster muscle tropomyosin expressed *in* Sf9 cells. *FEBS Letters, 394,* 201-205.