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<tr><td> </td><td>Experiment title: Influence of growth under microgravity on the diffraction quality of photosystem I crystals.<br>The structure elucidation of the membrane intrinsic protein complex photosystem I by X-ray crystallographic methods.</td><td>Experiment number:<br>LS-1449/LS-1464</td></tr>
<tr><td>Beamline:<br>ID14-EH2</td><td>Date of experiment:<br>from: 23 September 1999 to: 26 September 1999</td><td rowspan="2">Date of report:<br>30 August 2000<br>Received at ESRF:</td></tr>
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**Report:**

The aims of these experiments were to characterize the diffraction quality of photosystem I (PS I) crystals grown under microgravity during the STS-95 mission (experiment LS-1449) and to collect diffraction data of crystals containing a complex of PS I with its soluble electron acceptor ferredoxin (experiment LS-1464) suitable to solve the structure.

Crystallization under microgravity was done by dialysis using Advanced Protein Crystallization Facilities (APCF, European Space Agency). Six APCF reactors were used at slightly different salt concentrations of the reservoir solutions, resulting in PS I crystals in three of these reactors. Ground control experiments yielded crystals in two APCF reactors. Crystals were frozen in liquid nitrogen immediately after the landing of the space shuttle, in order to avoid extended degradation of the protein in the crystals. Partial data sets of ten different crystals grown under microgravity were collected, using a total rotation range of ~ 16° about the spindle axis of the goniometer. The needle shaped crystals were oriented with their hexagonal ***c*** axis roughly parallel to the spindle axis, and in a first step a special orientation was determined, where the crystallographic ***a*** axis is approximately parallel to the X-ray beam. In order to cover a comparable volume in reciprocal space for different crystals, data collections were started at –8° and stopped at +8° with respect to the special orientation. This symmetric rotation range with respect to the special orientation was chosen because of the twofold ambiguity in indexing of the reflections in the polar space group $P6_3$. The same strategy was applied to three crystals obtained from the ground control experiments.

The most significant difference between the crystals obtained from the microgravity experiments was the larger mosaicity of the ground control crystals (ranging from 1.5° to 2.5° as defined in DENZO/SCALEPACK) with respect to the microgravity crystals (ranging from 0.7° to 1.0°). As a consequence, the resolution limit observed for the ground control crystals was limited to ~ 4.0 Å because of the rejection of overlapping reflections during integration, although reflection maxima were visible to ~ 3.5 Å resolution. Comparably, crystals grown under microgravity diffracted to ~ 3.5 Å, as reflected by typical values for $<I>/<\sigma(I)>$ of ~ 3 at this resolution. Unit cell constants were comparable for both types of crystals ($a \approx 281$ Å, $c \approx 165$ Å). A general observation of this experiment is, that even the diffraction quality of the crystals grown under microgravity is clearly worse than that of the best crystals of PS I grown by the standard microdialysis procedure carried out under normal gravity (Fromme and Witt, 1998). One reason for this could be, that during the microgravity experiment the protein solution was kept at a relatively high temperature of 8°C about five times longer than in a normal crystallization experiment, presumably being responsible for a significant degradation of the protein, which results in less well ordered crystals. On the other hand, it should be taken into account that the limited number of APCF reactors which can be used in during a space shuttle mission results only in small numbers of microgravity experiments, less than statistically significant.

Two data sets of PSI/ferredoxin crystals were collected, one of them being 93.1 % complete from 25 to 8.4 Å resolution (91.4 % from 8.8 to 8.4 Å) with $R_{sym} = 0.082$ (0.331 from 8.8 to 8.4 Å) and $<I>/<\sigma(I)> = 14.5$ (2.5 in the highest resolution shell). The second data set was of slightly better quality (completeness 92.5 % from 25 to 8.0 Å resolution, 90.6 % from 8.4 to 8.0 Å, $R_{sym} = 0.078$ for total resolution range, 0.282 for highest resolution shell, $<I>/<\sigma(I)> = 16.2$, total range and 3.6 for highest resolution). Attempts to solve the structure by molecular replacement using the refined structure of PS I (Jordan et al., 2000) failed, possibly because the resolution is still too low.

**References:**

P. Fromme and H.T. Witt (1998) Improved Isolation and Crystallization of Photosystem I for Structural Analysis, *Biochim. Biophys. Acta* **1365**, 175-184.

P. Jordan, P. Fromme, O. Klukas, H.T. Witt, W. Saenger and N. Krauß (2000) Three-Dimensional Structure of Photosystem I from *Synechococcus elongatus* at 2.5 Å Resolution (in preparation).