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Report:

DATA COLLECTION

During this measuring period 25 crystals were used. Data were collected from 10, among them 20 were of H50S. Those contained (a) three native crystals mounted on spatula and loops, for comparisons); (b) eight soaked in $K_{14}NaP_5W_{30}O_{110}$ (W30), out of which three were suitable for data collection; (c) eight soaked in $((W_3O_2(O_2CCH_3)_6)^{+2}$ (WAC). Data were collected from four them. The other four were rejected since they showed clear unit cell deviations); and one soaked in Ta_6Br_{14}. The latter was diffracting to 3.2 A but due to shortage of beam time data were collection only to 6 A resolution. To minimize the time wasted for crystal screening, frozen pre-screened crystals, partially characterized at stations BW6 and BW7 at DESY, were used.

All experiments were performed at cryo temperature (about 90 K), using our home-built N_2 cryo-temperature equipment. Our preference for using this equipment is based on previous experience showing that our design is more suitable for ribosomal crystals than the commercial ones in terms of resolution limits and the maintenance of crystal life-time. Typical measuring time were 3 minutes (6 passes of 30 seconds) per rotation of half a degree. In average 6-8 exposures were needed for aligning the crystal and the determining the exposure time and searching for most suitable region(s) according to the following criteria: resolution, spot-shape, twinning and mosaicity.

For facilitating reliable data scaling, besides collecting the highest resolution shells (about 5-8 degrees from each fresh region), we continued to collect the lower resolution shells (down to 6-7 A) even after the crystals showed decay. The lower resolution reflections were instrumental during the course of merging data collected from different crystals or region, for constructing a data-set.

A way to increase the amount of high resolution data collected from a single crystal was to use larger crystals and irradiate specific regions of them. High quality crystal of a large size are more rare, since normally they tend to crack, split or grow with a high mosaicity. Two rather large crystals (axb about 500x300 microns) were selected for this experiment. Despite the use of crystals mounted in spatula, which significantly reduce their visibility while being irradiated, with some sophistication it was possible to translate from the beam-path the part of the crystal which was damaged and measure the parts which were not irradiated earlier. Using beam cross-section of 150x150 microns, these crystals could be exposed in four different locations. Once the decay of an exposed region passed the permitted limit, the crystal was translated, and a new region was exposed. It was found that the "fresh" regions were not harmed by the previous irradiation.

RESULTS

1. Data collection: the gain in resolution and exposure time: The most striking observation is the gain in resolution as well as in exposure time. Before their decay, all crystals checked at ID2 diffracted to higher resolution than that obtained from them BW6 or BW7 (Wiggler stations at DESY). Typically the limits were improved by 2-3 A (from 5-6 A to 3-4A). Occasionally even higher gain could be detected (e.g. from about 7 A to about 3.5 A). For obtaining the highest resolution, ID2 was faster than BW6 by 8-10 fold, from BW7 by 6-7 folds and from F1/CHESS by 3-4 folds. The comparison of the medium resolution is somewhat more complicated. For 1 degree rotation of the crystal soaked in Ta_6Br_{14}, the exposure time at ID2 was 5 minutes (6 A resolution), at BW6 around 20 min. (7A resolution) and at Fl/CHESS/CCD around 30 min. (4.75 A resolution).

2. Crystal decay: The crystals of H50S decay at ID2 even when irradiated while being kept at 85-90 K. The decay is manifested not only by the decrease in the resolution, but also by an increase in the mosaicity, the deterioration of the data quality as shown by the increase of the linear R-factors and the decrease in <I/Sigma > and by a significant change of the C-axis. At ESRF, during this beam time as well as in our test experiment (Nov 95) there were indications that the rate and severity of crystal decay depends on its initial resolution. Thus, the decay is faster for crystals diffracting initially to 3-3.5 A than for those diffracting initially to 4-5 A. In addition, beyond 6 A the rate of decay is slower even for crystals which diffracted originally to 3 A. Intuitively one may conclude that the lower the initial resolution the slower that decay, but as the crystals which diffracted to lower resolution were those derivatized by W clusters, it is also conceivable that the heavy atom clusters stabilize the crystals by an unknown mechanism.

3.: Although the resolution limits of the fresh crystals were higher that 3 A, the diffraction beyond about 4 A decayed within the first few exposures. Therefore only 5-8 degrees of high resolution diffraction could be collected from each crystal or crystal-region. It was found that despite the weak signal in this region, evaluation was possible, and the completeness (40-60%) and quality of the data at the farmost shell (34.1 A) are rather satisfactory. The data collected from different regions of the same crystals could be merged rather well, using for inter-scaling the medium and low resolution shells (6-20 A) collected before as well as after the decay (typical R(I) were around 10% for 90% completeness). Since when using the large crystal of the W30 derivative we continued data collection from each fresh region even after the highest resolution shell decayed, the completeness of the data of the W30 derivative at 5.5 A is around 85 % . Noteworthy is the comparison to other SR facilities. Thus, the best achieved at the second generation synchrotrons was 80-90% completeness to 7-9 A resolution with Rmerge(I) 12-15%. Attempts in merging data collected from different crystals have been rather successful despite the tendency of the ribosomes to form rather non isomorphous crystals. Thus, data from 4 out of 5 crystals soaked in W30 and 2 out of 3 soaked in WAC could be merged.

The crystals soaked in Ta_6Br_{14} provide an illuminating comparison. At Fl/CHESS data were collected on a 2K CCD detector (Princeton design) in slices of 0.1 deg. The collection time was controlled by counts. In average 2-3 minutes were needed per slice, leading to a total of 20-30 minutes per degree. At ID2, using the MAR detector, this strategy could not be employed, due to the length of time required for scanning and erasing the plate. Therefore rotation of 3 minutes per half a degree was chosen, which required a total of 6 minutes per degree. The gain in time at ID2 is obvious, However, for the weak reflections, the quality of the data collected in CHESS shows the superiority of short measurements. Thus, the <I/Sigma> obtained at ID2 is 2-3 whereas at CHESS the values were 8-10.

CONCLUSIONS and COMMENTS

For efficient performance some adjustments and fine tuning of the station were required. Since unexpected problems appeared (a crack in the intermediate nitrogen dewar) these operations required about half a shift in the beginning of our measuring time. During this beam time there were only a few minor failures, most of which have been fixed. These concern the stability of the beam position compared to the spindle position; the spindle slippage; the definition of the slits and the stability of the camera viewing the crystal and the quality of the image seen on it. Despite these problems, the feasibility of data collection from ribosomal crystals at the high brilliance station ID2 has been established and the superiority of this station over the second generation synchrotrons has been demonstrated.