NEW SAMPLE MOUNTING TECHNIQUE FOR PROTEIN CRYSTALS

The high-resolution cryogenic crystal structure of proteins obtained with brilliant synchrotron x-rays provides important information for studies on the mechanisms of their functions. However, it has been reported that the structure of proteins, particularly at their surface, may be altered when the proteins are cooled to cryogenic temperature [1]. For analysis under near-physiological conditions, it is necessary to collect diffraction data at room temperature. However, protein crystals are unstable at room temperature because of drying.

We developed an original crystal mounting method, namely, the humid air and glue-coating (HAG) method [2]. Protein crystals coated with a water-soluble polymer are stable at room temperature while humidity-adjusted air is blown on them (Fig. 1), enabling diffraction data to be collected at room temperature. The variation of lattice constants among individual crystals is relatively small; thus, this method is useful for multiple-crystal data collection. In addition, crystals coated under optimized humidity can be cryocooled without an additional cryoprotectant. Thus, the HAG method is also useful for high-resolution data collection at cryogenic temperature from fragile protein crystals that are readily affected by cryoprotectants (Fig. 2). It is a universal technique that will contribute considerably to the structure analysis of a wide range of proteins. The HAG method is available for users at the public beamline BL38B1.

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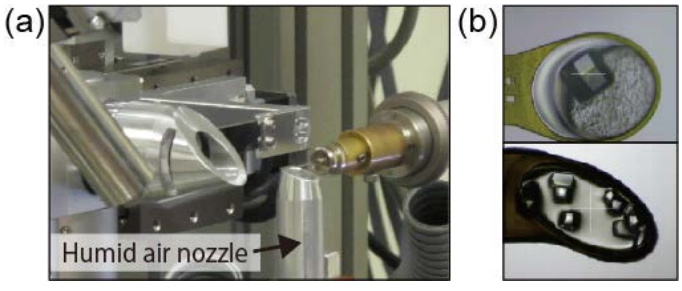

Fig. 1 Diffraction experiment using the HAG method.
(a) Equipment layout at BL38B1.
(b) Coated single crystal (upper) and multiple crystals (lower) after humidity adjustment.

References

[1] K. V. Dunlop, et al.: Acta Cryst. **D61**, 80 (2005).

[2] S. Baba, et al.: Acta Cryst. **D69**, 1839 (2013).

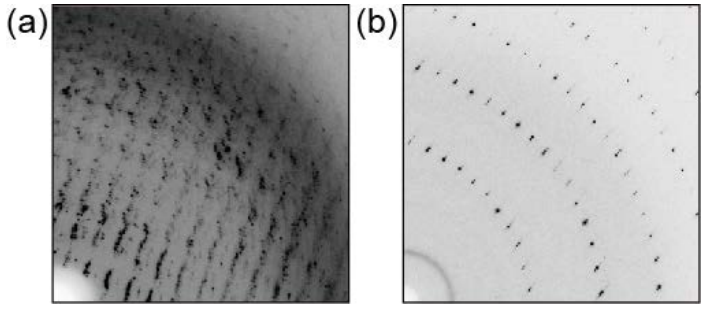

Fig. 2 Diffraction patterns from fragile protein crystals after flash-cooling.
Bacillus subtilis RsbQ crystals are mechanically fragile and split spots were observed when a cryoprotectant was used. By the HAG method, the cryoprotection of the RsbQ crystals became successful. (a) Diffraction pattern from a crystal when 25% (*v/v*) glycerol was used as a cryoprotectant. (b) Diffraction pattern from a crystal obtained by the HAG method.