Structure Determination of a Novel Protein by Sulphur SAD using Novel Crystal mounting Method

Yu Kitago, Nobuhisa Watanabe, Isao Tanaka, *Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo, Japan.* E-mail: kitago@castor.sci.hokudai.ac.jp

A crystal mounting technique was developed for the sulphur SAD method using longer wavelength X-rays. This technique is novel in that the a nylon loop is glued directly onto the tip of the micropipette and fixed as if the micropipette tip is located in the loop, so the solution caught in the loop can be aspirated through the micropipette just before flash freezing. Using this technique, the cryo-buffer and cryoloop can be removed easily before data collection to eliminate their X-ray absorption. The structures of novel proteins were solved using this technique in combination with chromium radiation. In the case of PH1109 from *P. horikoshii*, 90% of all residues were built automatically by *RESOLVE* using this technique, but only 76% were built for the dataset obtained using the standard loop. These results indicated that our crystal mounting technique was superior to the standard loop mounting method for the measurement of small anomalous differences, and yielded good results in sulphur substructure solution and phasing. We will demonstrate that the sulphur SAD method with a chromium source is more practical for macromolecular structure determination using our crystal mounting technique.

Keywords: sulphur, SAD, crystal mounting method