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USE OF RADICAL SCAVENGERS IN X-RAY DATA COLLECTION FROM PROTEIN CRYSTALS

OCCURRENCE of radiation damage is a feature normally encountered while collecting X-ray intensity data from single crystals of proteins. It is well-known that protein crystals contain large solvent regions consisting mainly of water. The protein crystals, like the molecules themselves, are stable only in aqueous medium and are therefore mounted with the solvent in a capillary for data collection. According to the indirect action theory of radiation effects, the damage to the protein molecule is associated with the production of free radicals from the aqueous medium. These radicals, in turn, transfer the energy acquired from the incident radiation to the protein molecule. The protein molecule is thus left in an unstable, ionized state leading to significant changes in its structure and properties. The onset of radiation damage is detected by reduction in the intensity of the diffraction pattern which proceeds on to its total disappearance on prolonged irradiation. The amount of radiation dosage required to cause the damage varies with substances, some affected within hours of irradiation and some others within few days. Hence, most often, in protein crystallography, one is compelled to use several crystals to collect a complete set of data. Most proteins are available only in small quantities and it is often difficult to grow a large number of good crystals. Also, correlation of different data sets sometimes poses problems when several crystals are used for data collection.

Some of the procedures adopted for alleviating the effects of radiation damage to protein crystals are given below :

(*i*) *Fast data collection* : The speed of intensity measurement can be improved by the simultaneous measurement of several reflections, either photographically[1] or by the use of multiple counters[2] or position sensitive detectors[3,4].

(*ii*) *Data collection at low temperature* : Even though protein crystals may be expected to be more stable at low temperatures, ice formation in the solvent regions and the consequent enhancement in the volume often disrupts the protein crystal structure. However, by working at high pressure and low temperature, the more suitable ice III-ice IX region of the water-ice phase diagram which does not involve any volume change could be chosen[5]. Also, by the choice of a proper solvent which on freezing forms a glassy medium instead of ice, the volume increase could be avoided[6].

We here suggest the use of radioprotectants as a means for controling radiation damage to protein crystals. A large number of substances are known to be potential radioprotectants. The protecting machanism of some of these compounds, in particular, the aminothiol group of protectants, has been attributed to their ability to react with the radiation induced free radicals. Therefore, if the radioprotectants are introduced in the protein crystal, radiation damage could be effectively reduced since the protectants compete for the radiation-induced free radicals. The free radicals are prevented from reaching the protein molecules as they are scavenged by the radioprotectant immediately after they are produced. Also, the radioprotectant after reacting with the radiation-induced free radicals do not themselves react with the protein crystal[7]. The radioprotectant could be easily diffused into the solvent region of the protein crystals by dissolving them in the mother liquor, during crystallization. The introduction of the radical scavengers in the protein framework is not expected to produce significant changes in the diffraction pattern of the crystal, as the former are generally low molecular weight compounds.

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