The SAXS studies of stability of glucose isomerase from *Streptomyces rubiginosus*

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Glucose isomerase (D-xylose ketol-isomerase, EC 5.3.1.5.) catalyses the isomerisation reaction of glucose and xylose. It is a homotetramer with the molecular weight of 172 kDa, made of four identical subunits [1]. Glucose isomerase is an exceptionally stable intracellular bacterial enzyme. It is used in industrial processes at temperatures of about 60°C with a half-life of hundreds of days [2]. The stability of solutions of glucose isomerase from *Streptomyces rubiginosus* on long-term storage and on exposure to synchrotron radiation has been studied by the small angle X-ray scattering (SAXS) method.

X-ray scattering data were collected at the beamline X33 [3] of the EMBL on the DORIS storage ring at DESY (Hamburg, Germany) using Mar-345 image plate detector. The sample-to-detector distance, was 2.6 m corresponding to the scattering vector range: $0.1 < s < 4.5$ nm$^{-1}$ (where $s=4\pi\sin\theta/\lambda$, $2\theta$ is the scattering angle and $\lambda=0.15$ nm is the X-ray wavelength). The detector’s range was calibrated using silver behenate. The protein samples of volume 100 µl were measured in 1 mm cells with mica windows (20 µm) at a temperature 15°C. The scattering data were collected in 300-s frames. The scattering of the buffer was subtracted and the final scattering curve was obtained by using the program PRIMUS [4]. To study the stability of isomerase solutions SAXS measurements were performed for glucose isomerase solutions prepared at 21, 14, 7, 5, 3, 1 days and directly before the measurements.

The values of $R_G$ for glucose isomerase varied from 3.24 to 3.31 nm, and the mean value was $R_G = 3.27 \pm 0.03$ nm. The variations in $R_G$ (± 1.0 % of the mean value) are not suggestive of changes following from aggregation or degradation of proteins on long storage. Radiostability of glucose isomerase has been also evaluated on the basis of comparison of selected parameters characterising fresh and irradiated samples. The values of the gyration radius are similar: $R_G=3.28$ nm and $R_G=3.26$ nm for the fresh and irradiated samples, respectively. The difference in the forward scattering ($I(0)$) values reaches 1.2 %. Therefore glucose isomerase is a good candidate to replace BSA as a molecular weight standard in the SAXS measurements.

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References