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Analysis of polypeptide chain conformation in 8M urea by SAXS technique

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Introduction

Protein polypeptide chain in strong denaturant solutions have the conformation of random coil approaching in ideal case to Gaussian coil conformation. In ideal solvent the dimension of coil is proportional to $M^{0.5}$. In good solvents the power is between 0.5-0.8. Study of unfolded proteins is the first step to elucidate a protein self-organisation and shed light on the conformation of partially folded proteins. Much information on protein conformation can be extracted from SAXS patterns. For Gaussian coil the scattering factor P(q) is given by formulae: $P(x)=2*(x-1+\exp(-x))/x^2$, where $x=(q*R_g)^2$ with R_g- radius of gyration and q- module of scattering vector[1]. Unfortunately, the Guinier region for R_g evaluation of Gaussian coil is very small ($x<0.3$) causing the difficulties with precise its measurement due to intermolecular interference influence on the SAXS pattern. But the region for R_g evaluation can be increased up to $x<12$ with about 0.5% accuracy according to approximation: $1/P(x)=1+0.36*x^{1.1}$ derived from previous formulae upon Taylor series expansion. It permits to calculate R_g value from part of SAXS pattern not disturbed by intermolecular interference. We checked this approach for several proteins in 8M urea.

Experimental

For SAXS experiments yeast phosphoglycerate kinase (PGK, M=44.7 kDa), bovine carbonic anhydrase B (CAB, M=29.1 kDa) and hen egg white lysozyme (HEWL, M=14.3 kDa) were used in 50mM Na-phosphate (pH7), 50mM NaCl buffer with 8M urea. The initial concentrations were 10-15 mg/ml. To disrupt S-S bonds in lysozyme 20mM of DTT was added to protein solution. Synchrotron X-ray measurements were done on a small-angle camera BL-6A (Photon Factory, Tsukuba) using CCD-detector. The range of scattering vectors Q=0.008-0.2 $Å^{-1}$.

Results

SAXS pattern for R_g evaluation of PGK in 8M urea is presented in Fig.1. On inset of Fig.1 the experimental SAXS patterns at different concentrations are given. One can see the strong concentration dependence of SAXS patterns at small scattering angles. SAXS data plotted from the region indicated by rectangle show good linear dependence. The dependence of evaluated R_g values for three proteins on their molecular mass is presented in Fig.2. The slope is 0.65 which corresponds to proteins in good solvent.

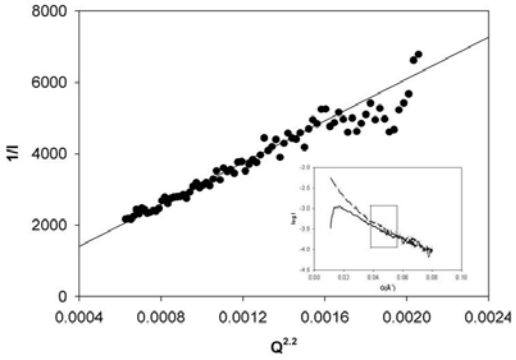

Fig.1 SAXS pattern for PGK R_g evaluation. Inset: SAXS patterns at different concentrations. Rectangle points out the region for R_g evaluation.

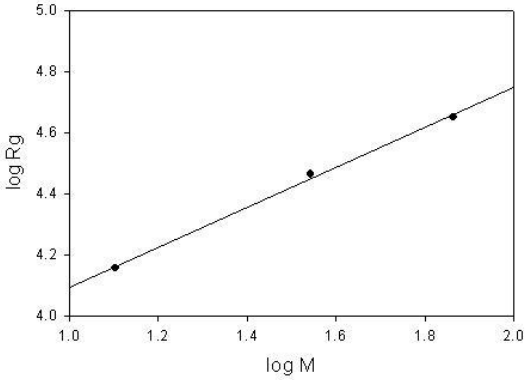

Fig.2 Dependence of R_g on molecular mass M for HEWL, CAB, PGK. The slope is 0.65.

References

1) O. Glatter, O. Kratky, in: "*Small Angle X-ray Scattering*", Academic Press, London 1982, p.1-515

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