ESRF	**Experiment title:** Perturbative effects on structural intramolecular rearrangements of alpha-crystallin in water solution.	**Experiment number**: SC-3271
Beamline: ID14-3	**Date of experiment**: from: 23/09/2011 to: 26/09/2011	**Date of report**: 23/02/2012
Shifts: 9	**Local contact(s)**: Petra Pernot	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

* VILLARI Valentina, CNR-Istituto Processi Chimico-Fisici, Viale F. Stagno d'Alcontres 37, I-98158 Messina

* MICALI Norberto, CNR-Istituto Processi Chimico-Fisici, Viale F. Stagno d'Alcontres 37, I-98158 Messina

ATTANASIO Francesco, CNR-Istituto di Biostrutture e Bioimmagini, Viale A. Doria 6, I-95125 Catania

Report:

Small angle X-ray scattering intensity profile of buffered (20 mM phosphate buffer, pH= 7.2) aqueous solutions of α-crystallin protein at a concentration of 10mg/ml was investigated at 25°C and 60°C. α-Crystallin is the major structural lens protein and belongs to the heat shock protein family; it is known to act as molecular chaperone, thus being crucial in protecting various proteins against aggregation induced by heating, chaotropic agents, reduction, and chemical modification. In order to study the effect of small molecules on the structure or size of α-crystallin (through heating) and their influence on the chaperone activity against aggregating target proteins, the scattering profile was also measured for α-crystallin buffered solutions in the presence of carnosine (50 mM) and guanidine (1 M). SAXS data were also collected at 25°C after heating the solutions at 60°C.

The obtained data for α-crystallin protein in water and in the presence of carnosine and guanidine at room temperature, normalized to zero-angle scattering intensity I(0)=1 are shown in Figure 1. The obtained curves were subjected to Fourier transform, after which pairwise distance function *P(r)*, radius of gyration R_g and maximum macromolecule dimension D_{max} were calculated. The obtained results are shown in Figure 2 and Table 1.

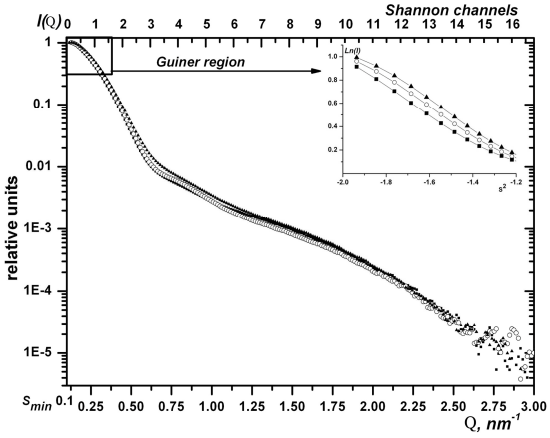

Figure 1. Small angle scattered intensity for α-crystallin solutions at 10 mg/ml in: ○ buffer; ■ buffer with guanidine; ▲ buffer with carnosine. T=25^0 C.

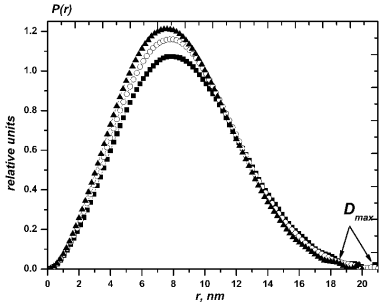

Figure 2. Pairwise distance functions for α-crystallin derived from Fig.1 data (symbols are the same as in Fig. 1), determined using GNOM program.

Table 1.

Sample	Geometric parameters	
	R_g, nm	D_{max}, nm
○	6.7 ÷ 6.8	20.0 ÷ 20.5
■		
▲	6.5 ÷ 6.6	19.5 ÷ 20.0

The obtained data for α-crystallin protein in water at 25° and 60°C are shown at Figure 3. The pairwise distance function *P(r)*, radius of gyration R_g and maximum macromolecule dimension D_{max} were calculated and the obtained results are shown at Figure 4 and Table 2.

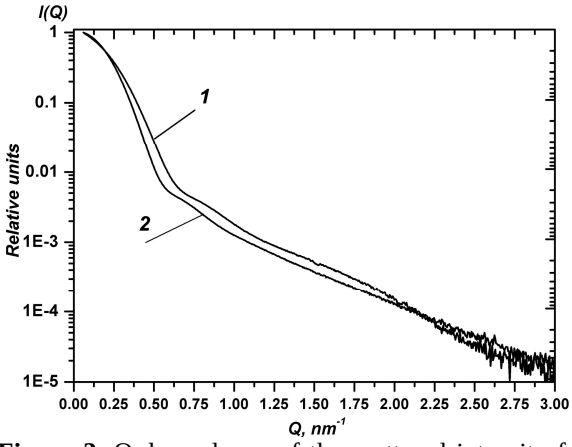

Figure 3. Q-dependence of the scattered intensity for α-crystallin solutions at 25° (1) and 60°C (2).

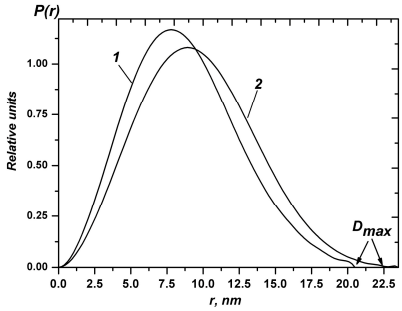

Figure 4. Pairwise distance functions for α-crystallin derived from Fig.3 data, determined using GNOM program.

Table 2.

Sample	Geometric parameters	
	R_g, nm	D_{max}, nm
1 (25^0C)	6.7 ÷ 6.8	20.0 ÷ 20.5
2 (60^0C)	7.3 ÷ 7.4	22.5 ÷ 23.0

SAXS data obtained at 25°C and 60°C for oligomeric α crystallin in water solution were used to calculate the molecular shape using finite volume element modeling, assuming P_{10} symmetry[1], by GASBOR 22 program. The algorithm is based on the task of searching for a spatial distribution of finite unitary elements. The latter can be closely-packed spheres, i.e. atoms of defined radius $r_0 << D_{max}$ [2] or a set of amino acid residues [3]. Simulated annealing method [4] with additional restraints (compactness and continuity of the generated set) is used as a basic method of sampling possible shapes. During the search, the optimization program randomly inverts the density of a given unitary element (changes it from particle density to solvent density or vice versa). A modeled small angle scattering curve $I_{mod}(Q)$ is calculated according to Debye formula after each structure modification step. The resulting protein shape obtained with this method is shown on Figures 5a and 5b.

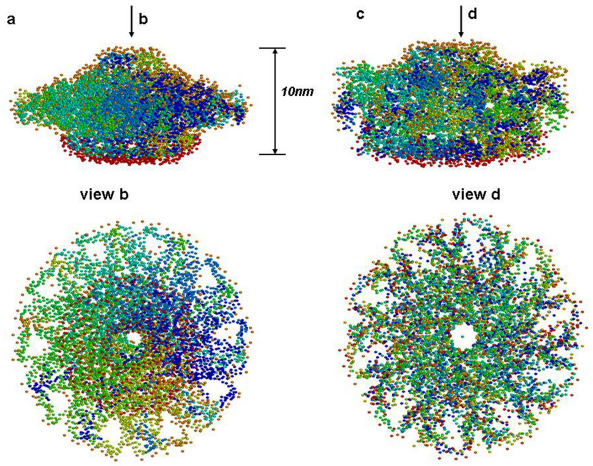

Figure 5. α-crystallin model composed of 6246 amino acid residues, considering a 3:1 ratio αA/αB-crystallin; a) 25°C, b) 60°C.

Analogous calculations from the SAXS data for α-crystallin at 60°C and at 25°C after temperature annealing in the presence of carnosine and guanidine are in due course.

References

[1] B. Groth-Vasselli, T.F. Kumosinski, P.N. Farnsworth, *Computer -generated model of the quaternary structure of alpha crystallin*, Exp. Eye Res. (1995) **61**: p.249-253.

[2] Svergun D.I. *Restoring low resolution structure of biological macromolecules from solution scattering using simulated annealing.* Biophys. J. 1999. **V.76**: p.2879-2886.

[3] M. Petoukhov , D. Svergun, *New Methods for domain structure determination of proteins from solution scattering data*. J. Appl. Cyst. (2003) **36**, p.540-544.

[4] D.I. Svergun, *Restoring low resolution structure of biological macromolecules from solution scattering using simulated annealing.* Biophys. J. 1999. **V.76**: p.2879-2886.