Protein-protein interactions leading to amyloid fibrils

M.G. Ortore[1], R. Sinibaldi[1], F. Spinozzi[1], T. Narayanan[2], A. Shukla[2], S. Villasi[3], G. Irace[3] and P. Mariani[1]

1. Dipartimento di Scienze applicate ai Sistemi Complessi, Università Politecnica delle Marche, Ancona, Italy

2. European Synchrotron Radiation Facility (ESRF), Grenoble, France

3. Dipartimento di Biochimica e Biofisica, Seconda Universit degli Studi di Napoli, Italy

The issue of protein aggregation represents a wide ensemble of results from biological, biochemical and biophysical studies carried out to date. Under some pathological conditions, a protein converts from its soluble form into highly ordered aggregates that are generally referred to as amyloid fibrils. Amyloid aggregates are until now associated with 40 human diseases [1]. Although the details of the relationship between protein accumulation and degeneration are not clear, it is thought that the aggregation process plays a key role in impairing cellular function, leading to death of involved cells. In order to tackle the prevention and treatment of these diseases, it is crucial to understand the mechanism and molecular details of the pathological aggregation of proteins into fibrillar structures. In vitro amyloid fibril formation is preceded by the formation of metastable non fibrillar forms: prefibrillar aggregates which appear as globules 2.5-5.0 nm in diameter or larger, which are responsible for cytotoxicity underlying neurodegeneration. The molecular mechanisms leading intrinsically disordered globular proteins and / or membrane proteins into prefibrillar aggregates are still unclear, although recent studies, looking for the understanding of protein aggregation path, focus mainly on the existence of sequence determinants [2].

We performed a set of SAXS experiments on the apomyoglobin mutant W7FW14F, that at physiological pH and room temperature, firstly aggregates in prefibrillar forms that are cytotoxic and then forms amyloid fibrils [3]. The first stages of W7FW14F oligomerization are induced by a pH jump from 4.0 to 7.0, but it was not known if the aggregation of W7FW14F apomyoglobin mutant starts from a native-like state or from a partially folded species. Spectroscopic data suggested that the secondary structure of W7FW14F apomyoglobin at pH 4.0 was just slightly different from the one occurring in the native state of wild type protein. SAXS experiment evidenced that at pH=4.0 the mutant apoMb is in a compact globular state, too (Fig.1).

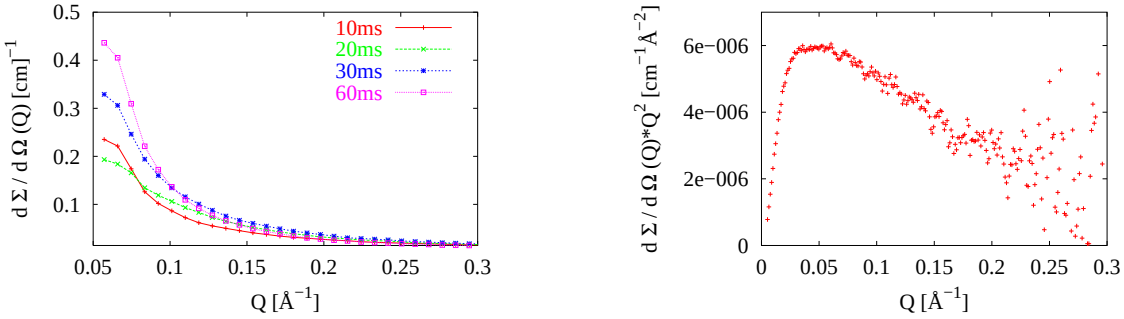

Fig. 1 Left. Experimental SAXS curves referring to W7FW14F apoMb during a pH jump. Spectra correspond to the evolving of protein aggregation starting from 10 ms after mixture up to 60 ms. Right. Kratky plot of W7FW14F apoMb at pH 4.0. The bell-shaped form verifies the presence of compact and globular objects in solution.

SAXS experiment has been performed at ID02 beamline with a stopped-flow equipment and it has been possible to follow protein aggregation kinetic despite the fast reate of oligomerization process, since protein solution pH value has been varied from 4.0 to 7.0 in less than 10 ms. SAXS data (Fig. 1) show that pH modification induces big changes in W7FW14F in solution in a very short times range. The extrapolated intensity at $Q = 0$ does not follow a trend versus time, but this behaviour can be explained in terms of a structure factor which cannot be neglected in the first aggregation steps, when monomer population gives rise to a bigger number of interacting particles. Nonetheless, the pattern of protein prefibrillation can be estimated considering the presence of an unknown number of different aggregates populations. Singular Value Decomposition (SVD) of the data [4] yields a set of functions, from which all the scattering curves can be reproduced using appropriate coefficients. The major result of our study is the determination of the presence of three different oligomers and their relative weight in each step of protein prefibrilation. Finally, time-resolved SAXS experiments together with the estimation of different oligomers via SVD method, can be considered a new and useful approach to investigate the first stages of amiloidogenic processes.

References

[1] F. Chiti, and C. M. Dobson,"Protein Misfolding, Functional Amyloid, and Human Disease," Annu. Rev. Biochem. **75**,333 (2006).

[2] M. Lopez de la Paz, and L. Serrano, "Sequence determinants of amyloid fibril formation," Proc. Natl. Acad. Sci. USA **101** , 87 (2004).

[3] C. Malmo, S. Vilasi, C. Iannuzzi, S. Tacchi, C. Cametti, G. Irace, and I. Sirangelo, "Tetracycline inhibits W7FW14F apomyoglobin fibril extension and keeps the amyloid protein in a prefibrillar, highly cytotoxic state," FASEB **20**,346 (2006).

[4] E. R. Henry J. Hofrichter. "Singular value decomposition: Application to analysis of experimental data," Meth. Enzymol. **210**,129 (1992).