15A/2002G329

Studies on size and shape variation in alpha crystallin and its mutants using small angle X-ray scattering

Ch. Mohan RAO*[1], Volety SRINIVAS[1], Saloni PASTA[1], Ryohei YOSHIDA[2], Tomonao INOBE[2], Kohske MAKI[2], Kiyoto KAMAGATA[2], Sawako ENOKI[2], Akio KADOOKA[2], Munehito ARAI[3], and Kunihiro KUWAJIMA[2]

[1]Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad-500007, India
[2]Dept. of Physics, Univ. of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[3]IBRF, AIST, Tsukuba, 305-8566 Japan

Introduction

Protein misfolding and aggregation are shown to be the molecular basis for a growing number of diseases. Molecular chaperones and heat shock proteins help the cells in dealing with misfolding and aggregation. α-Crystallin is an eye lens protein with significant homology with small heat shock proteins. In addition to eye lens, α-crystallin is constitutively expressed in several other tissues such as heart, kidney and the brain. Interestingly its expression is up regulated in disease conditions. Expression of α-B crystallin is enhanced in cerebral cortex of Alzheimer's patients. Point mutations in α-crystallin (R116C in αA-crystallin and R120G in αB-crystallin) lead to congenital cataract and desmin related myopathy.

Alpha crystallin constitutes of two gene products αA-, and αB-crystallins and has a molecular weight of approximately 600-800 kDa. This is highly polydisperse and difficult to crystallize. Studies from many laboratories, including our own, show that α-crystallin possesses chaperone activity in preventing aggregation of other proteins and refolding enzymes to their active state. Based on several lines of experimental results from our laboratory, we believe that the oligomer size and shape, in addition to the surface hydrophobicity and appropriate distribution of charges, influence the chaperone activity. Keeping this in mind, several mutants of αA-crystallin (αAdel, ABce, R116C, αAIXV) and αB-crystallin (αBdel, BAce, R120G, αBGXG) have been made and their chaperone activity measured. SAXS analysis of the wild type recombinant proteins and the mutant molecules would be useful to determine their size and shape and could be correlated with the chaperone activity.

Experimental

All the proteins were overexpressed in *E.coli* and purified. For SAXS measurements of the proteins, 10 mM Na phosphate buffer (pH 7.2) containing 100 mM NaCl was used. Scattering patterns were recorded at 25 °C using a CCD-based X-ray detector with 2.3 m sample-to-detector distance. Scattering patterns of all the proteins were measured as a function of concentration, in the range between 0.5 mg/ml to 2 mg/ml. The raw SAXS data were corrected for intrinsic image distortion, non-uniformity of response and contrast reduction. The R_g and molecular weight values were estimated by the Guinier approximation.

Results and Discussion

SAXS data of wild type αB-crystallin gives a molecular weight of 421 ± 15 kDa which is in agreement with the literature value, measured by different techniques. However, the molecular weight of αA-crystallin as calculated from SAXS data (350 ± 21) is different from the literature value of about 600 kDa and this needs to be verified. The mutant, BAce, generated by swapping the C-terminal extension of αA-crystallin with that of αB-crystallin displays higher chaperone activity than either of the wild type proteins. SAXS data shows a decrease in the R_g and molecular weight values of the mutant BAce (R_g =52.7 ± 0.3, MW=258 ± 7) in comparison with either αA-wild type (R_g = 53.9 ± 0.6, MW =350) or αB-wild type (R_g =54.3 ± 0.3, MW = 421 ± 15). This decrease in molecular weight correlates well with enhanced chaperone-activity shown by this molecule. In contrast, swapping the C-terminal extension of αB-crystallin with that of αA-crystallin results in the mutant ABce and this mutant has a considerably decreased chaperone activity than either of the wild type proteins. SAXS data of this mutant ABce shows an expected increase in its molecular weight. Thus the SAXS data lends credence to the suggestion that oligomer size plays a role in the functionality of the heat shock proteins. The mutants, αAdel and αBdel display enhanced activities compared to the respective wild type proteins. SAXS data suggests a marginal decrease in their respective molecular weights (303 kDa and 414 kDa) compared to the wild type molecules (αA -350 kDa and αB -421 kDa). Our results with the mutants R120G and R116C remain inconclusive and need further investigation using SAXS. We have shown earlier that chaperone activity of alpha crystalline is temperature dependent and charges play a crucial role on the oligomer size. SAXS measurements of all these proteins as a function of ionic strength and also as a function of temperature would give further insights into the mechanistic aspects of alpha crystallin

* mohan@ccmb.res.in