MS04-P05

Protein misfolding in chimeras of the sh3 domain of the c-src and fyn tyrosine kinase

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In the next few years, due to the increase in life expectancy, neurodegenerative diseases, as for example the Alzheimer's disease, will be one major health problem in the developed countries. This disease belongs to the group of those caused by protein misfolding associated with the development of amyloid deposit. The study of the molecular basis of the missfolding processes is complicate due to the difficulties to obtain structural information at atomic level of such class of oligomers. However some proteins suffer misfolding forming oligomers which are able to crystallize. This oligomerization process is known as 3D domain-swapping (3D-DS), and the interchage of secondary structure elements may result in the formation of dimers, trimers, etc[1]. A case of study is the SH3 domain of the c-Src tyrosine kinase which develop intertwined dimers and, also, is able to form amyloid fibers. The formation of these 3D-DS structures and amyloids is pH dependent and very responsive to some puntual mutations[2]. Considering the results obtained in previous studies, our group have clonned, expressed and purified several chimeric proteins of the SH3 domain of the c-Src and Fyn tyrosine kinase, where the RT and n-Src loops of each domain have been interchanged[3]. In the c-Src SH3 the n-Src loop acts as hinge loop, which facilitate the opening of the domain to form the intertwined oligomer. We have study the stability of these proteins vs the pH (pH range 1-14) and in presence of the chemical denaturant guanidinium hydrochloride. These chimeric proteins are stable in a broad range of pH (pH 5.0-11.0) and show a high stability. The stability is higher at neutral pH than at acidic pH values. We have determined the oligomerization of the proteins by dynamic light scattering. The formation of dimers at acidic pHs is favoured by the addition of low molecular weight PEGs. Considering these results, we have screened the crystallization conditions at different pHs and in presence of several additives that promotes the formation of the dimers. Here we present the preliminary biophysical characterization of these chimeric proteins and their crystal structures determined at several pH values and oligomeric states. The results obtained show that besides residues at the hinge loops some residues al the distal loop might play a key role in the opening of the protomer to form the intertwined oligomers.

References:

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Crystal structures of chimeric sh3 domains forming 3d-domain swapping in presence of urea

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Amyloid forming proteins are the main cause of many neurodegenerative diseases. However, the mechanism of their formation at molecular level is not known. Tackling this task is difficult because the limitations to obtain high resolution structural information. We have constructed several chimeric proteins of the SH3 domains of the tyrosine kinases c-Src, Abl and Fyn as model proteins to tackle these studies. In those SH3 domains the RT and n-Src loops of each domain have been interchanged. Previous studies have demonstrated that some of these chimeric proteins are able to form oligomers by 3D domain-swapping (3D-DS), and the interchage of secondary structure elements results in the formation of dimers[1,2]. Structural studies performed with proteins under conditions near denaturation might help to understand the initial steps which drives misfolding in proteins. Considering this approach, we have studied the binding of the chemical denaturant urea to several chimeric constructions of the c-Src SH3 domain. We have crystallized these proteins in presence of different concentrations of urea to determine its interaction. Previously, we have analyzed the unfolding of the chimeric proteins in presence of urea at pH 5.0 and pH 7.0 by means of the intrinsic fluorescence of the protein. Some chimeras of the Src-SH3 where the RT and n-Src loops have been interchanged by those present in the Fyn SH3 domain (SF chimeras) does not fit a two-state model, which might indicate the presence of some intermediate in the unfolding process as it has been described in other SH3 domains[3]. Most of the crystals grown in presence of urea diffracted to atomic resolution, which allowed us to model the urea molecules interacting with residues at the surface of the protein. In all the structures solved the urea molecules were modelled replacing first-shell water molecules, which would modify the water-water hydrogen bond network. We have analyzed the results considering structural changes due to urea binding: in the position of the protein main chain; rotamer side chain changes; and, displacement of buried water molecules. In some structures a urea molecule is placed interacting with Glu106, which is critical in the nucleation of the folding process of the c-Src-SH3 domain by forming a hydrogen bond with Ser123.

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