

α -synuclein Aggregation Vs. β -synuclein Aggregation Inhibition: The Strange Case of Dr. Jekyll and Mr. Hyde of the Protein World

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Abstract. α -synuclein has been recognized as the main proteic agent causing neurodegenerative diseases, e.g. Parkinson's disease. Many studies suggest that β -synuclein acts as an inhibitor of α -synuclein aggregation, controlling its toxicity. Action mechanisms of β -synuclein, specifically its inhibition capabilities, are poorly understood. In this work, we use *in silico* methodologies to elucidate some structural and dynamical features of β -synuclein, when immersed in a physiologically representative environment. Our results confirm the conclusions of Bertoncini *et al.* about the level of disorder of β -synuclein, which is above 95 %. Also, our study highlights relevant features about the statical and dynamical conformational variability of β -synuclein, with emphasis in stability and surface characterization.

Keywords: Synuclein · Molecular Dynamics · Disorder.

Introduction

It has been suggested that α -synuclein (α -s) protein is the main agent in Parkinson's disease (PD).[1] Changes in the molecular structure of α -s causes the formation of agglomerates, that later accumulate in the brain, specifically in the *substantia nigra*, causing the characteristic signs of Parkinson's disease, such as bradykinesia, muscle rigidity, tremor and altered reflexes.[1, 2] It has been found that β -synuclein (β -s) [3], which is a homologous protein to α -s, is also present in various areas of the brain in patients with PD, including the *substantia nigra*, suggesting that β -s also participates in the degenerative process. However, other studies suggest that this protein could acts as an inhibitor of the aggregation process of α -s, counteracting its toxicity.[3,4]

The action mechanism of β -s of either the aggregation process or the aggregation-inhibition process of α -s is still unknown, despite the efforts of many researchers who have tried to elucidate the processes of interaction between the two structures.

On the other hand, β -s and α -s proteins belong to the group of the Intrinsically Disordered Proteins (IDPs).[4] This type of proteins represent a turning point at the established and accepted paradigm structure-function, which has been a long accepted fact in the field of molecular biology.[5] We refer here to the standard procedure that has been considered for the understanding of the folding process of proteins:

Sequence DNA - Sequence RNA - Sequence AA - Structure 3D - Function

where the prediction of the *Sequence AA - Structure 3D* is called the protein folding problem, and where the *Sequence AA - Structure 3D - Function*, is the paradigm of the structure and protein function generally accepted.[5, 6]

From the point of view of structural biology, IDPs set an enormous challenge, since they are conformationally extremely heterogeneous, and, therefore, their structures are not easily determined by standard techniques.[7]

Table 1. General alignment of the primary structure of the β -s and α -s proteins. Highlighted in blue and red are the amino acids in which both proteins differ. Dashes represent the absence of amino acids in the sequence.

										10										20										
α -s	M	D	V	F	M	K	G	L	S	K	A	K	E	G	V	V	A	A	A	E	K	T	K	Q	G	G				
β -s	M	D	V	F	M	K	G	L	S	M	A	K	E	G	V	V	A	A	A	E	K	T	K	Q	G	G				
										10										20										
30										40										50										
α -s	V	A	E	A	A	G	K	T	K	E	G	V	L	Y	V	G	S	K	T	K	E	G	V	V	H					
β -s	V	T	E	A	A	E	K	T	K	E	G	V	L	Y	V	G	S	K	T	R	E	G	V	V	Q					
30										40										50										
60										70										80										
α -s	G	V	A	T	V	A	E	K	T	K	E	Q	V	T	N	V	G	G	A	V	V	T	G	V	T					
β -s	G	V	A	S	V	A	E	K	T	K	E	Q	A	S	H	L	G	G	A	V	F	S	-	-	-					
60										70										80										
80										90										100										
α -s	A	V	A	Q	K	T	V	E	G	A	G	S	I	A	A	A	T	G	F	V	K	K	D	Q	L					
β -s	-	-	-	-	-	-	-	G	A	G	N	I	A	A	A	A	T	G	L	V	K	R	E	E	F					
80										90										100										
110										120										130										
α -s	G	-	-	-	K	N	E	E	G	A	P	Q	-	-	E	G	I	L	E	D	M	P	V	D	P					
β -s	P	T	D	L	K	P	E	E	V	A	Q	E	A	A	E	E	P	L	I	E	P	L	M	E	P					
100										110										120										
130										140										150										
α -s	D	N	E	A	Y	E	M	P	S	E	E	G	Y	Q	D	Y	E	P	E	A										
β -s	E	G	E	S	Y	E	D	P	P	Q	E	E	Y	Q	E	Y	E	P	E	A										
120										130										140										

Bertoncini et al. [8], for example, used Nuclear Magnetic Resonance (NMR) to elucidate the structural and dynamic characteristics of the soluble state of the β -s protein as a first step to understand the molecular basis of its anti-amyloidogenic effect on the α -s. Their study shows that β -s protein adopts an extended conformation in their native state, in addition to shows transient conformations of polyproline II helix (PPII) grouped in the C-terminal region.[8] The PPII helix is a secondary structure that is present in proteins with a re-

peated number of proline residues. This secondary structure is characterized by having dihedral angles (ϕ, ψ) , which give the proteins a certain structural conformation and contribute to protein-protein and protein-nucleic acid interactions, because it is principally found at domains binding sites.[9] It has been suggested that the presence of this repeated number of proline residues in the C-terminal region of β -s, which are not present in α -s [Table 1], could be a key piece for the inhibitory role of β -s on the aggregation of its homologous protein.[8]

However, characterization of the behavior of the IDPs requires new approaches with respect to the standard procedures for determining structures. Computational methods offer a wide range of tools that might allow us to look at an atomistic level how the aggregation process occur. One of the main techniques used to study these type of processes is Molecular Dynamics (MD).[10, 11] MD allows us to study how atoms and molecules interact during a given period of time, offering a trajectory of the dynamic evolution of the system in which the conditions of the same can be varied.[10,12] The trajectories of these atoms and molecules are based on the equations of classical mechanics, or Newton's equations of motion.[12]

1. The law of inertia: *"Every body continues in its state of rest or uniform motion in a straight line unless it is compelled to change that state by forces impressed".*[13]. Namely, if

$$F = 0 \quad (1)$$

then

$$m \times v = \textbf{constant} \quad (2)$$

2. The fundamental equations of dynamics: *"Mutation en motion is proportional to the motive force impressed, it is a straight line where the force is impressed".*[13] The motion of the particle is described by the differential equation:

$$F = \frac{dp}{dt} \quad (3)$$

where

$$p = m \times v \quad (4)$$

In most cases, the mass of the particle is constant so this equation can be reduced to:

$$F = m \times a \quad (5)$$

where a is the particle acceleration vector

$$\sum_{n=0} F_{ji} + F_i^e = p_i \quad (6)$$

where

$$F_i^e \quad (7)$$

is the external force and

$$F =_{ji} \quad (8)$$

is the internal force exerted by the j -th particle over the i -th particle.[14]
Now, MD uses the equations of motion to describe a system of particles, so the equation would be the following, taking as an example the i -th particle:

3. Interaction law "*The forces that exert two bodies on each other are always equal in opposite directions*".[13]

$$F_{ij} = -F_{ji} \quad (9)$$

where

$$i \neq j \quad (10)$$

β -s protein is an IDP, namely, it doesn't have a defined tertiary structure.[3, 8] For this species, MD provides the most appropriate tool to study the protein-protein and protein-environment phenomena, thus, contributing to the understanding of the possible mechanisms of action of the β -s to inhibit the aggregation processes of α -s.[14, 15]

In the present work, we intend to contribute to the understanding of the factors that intervene in the process of inhibition of α -s by the β -s protein, initially by studying the structure of the β -s protein and its dynamics, within a physiologically representative environment.

Methodology

1. Use of the DisProt[16] disordered structures database to obtain the primary structure of β -s protein, which will be used later to obtain secondary structures.
2. Dilucidation of the possible secondary structures of the protein using prediction software's for secondary structures and disordered secondary structures, e.g. *SSpro* and *SSpro 8-sx* protein predictor[16], the *s2D* method[17], PSIPRED, DISOPRED3[18] and PredictProtein software's.

On the other hand, we use prediction software for tertiary structures and prediction software for disordered tertiary structures with the aim of obtaining possible 3D structures of β -s and thus be able to make structural comparisons with α -s, in addition to performing the MD simulations.

This step is done because the β -s protein doesn't have a defined secondary structure, and therefore, it's not accessible from on-line databases.

3. Use of *ab initio* methods, templates-based, sequence-based, meta-predictors and homology methods for the determination and quantification of structural disorder that takes into account the mathematical algorithms on which the software is based, to carry out the prediction. Conventional structure prediction software is not suitable for this type of proteins because they do not take into account the possible areas of the primary sequence of amino acids that can present disorder.

4. The determination of the protonation states of the amino acids for each selected structure will be carried out with the use of free software. Once the protonation states have been determined, the construction of the simulation cells is carried out.
5. The simulation cells are constructed taking into account physiological conditions, such as pH and temperature. The Gromacs 5.1 software is used to obtain the DM trajectories.

Results

We obtained eight possible tertiary structures of β -s protein [Fig. 1] from four predictors of secondary structures. The eight structures obtained present a lot of difference at the conformational level, which suggests that the interactions of the protein-protein and protein-environment type can vary repeatedly.

Partial results confirm the approaches of authors such as Bertoncini and collaborators about the percentage of disorder that presents the β -s protein, which is above 95 %, in addition to the presence of PPII helix residues, mainly in the C terminal region [Fig 2]. This suggest that β -s is a unstable protein and have a high probability of interaction with other substances. In this case, this interaction could explain the inhibitory action over the toxicity of α -s protein.

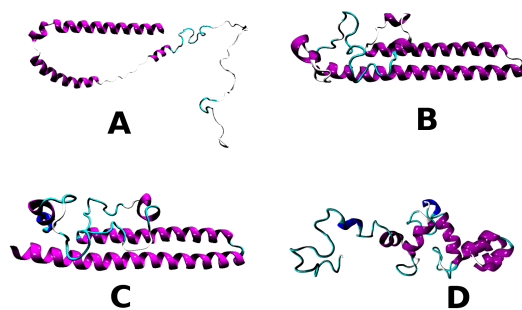


Fig. 1. Structures of β -s protein from predictors of secondary structure, here we present four of the eight structures obtained. **A.** The molecule present three alpha helix, two long and one short, also a large chain without structure. **B.** This structure has two large alpha helix that are more coupled and an area that has no defined structure. **C** Here, the molecule has two large alpha helix, a chain without structure and a small secondary structure of beta helix. **D** This structure has a very short chain of alpha helix, a large disordered chain and a small chain of beta helix.

On the other hand, the β -s protein do not have a large number of hydrophobic amino acids and cysteines. These amino acids confer to molecular structures

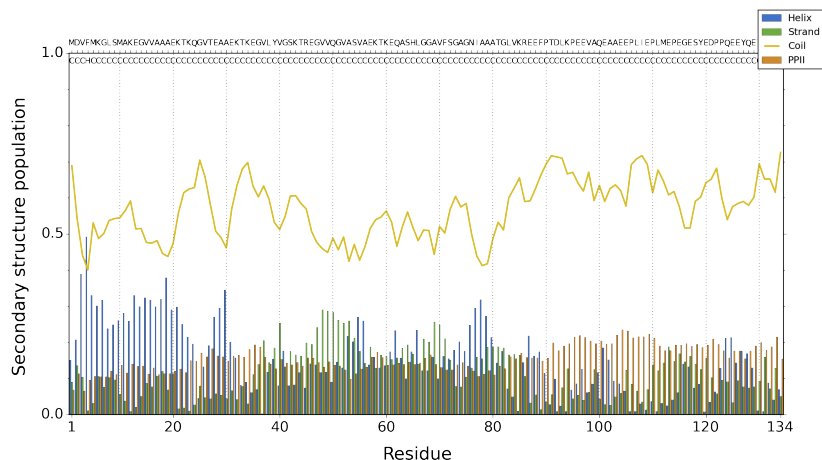


Fig. 2. Prediction of secondary structure and relative disorder levels of β -s protein with *s2D* software. The secondary structure of the protein consists mainly of alpha helices. The regions of the molecule that do not have structures are the largest representative of the structural disorder of the protein. The PPII residues are shown mainly in the C terminal region. The disorder level of the structure is measured in a range of 0 - 0.5 - 0.1, following the algorithms used by the software. With a level of disorder above 0.5, the protein is considered disordered.

much of its stability. Our study show relevants characteristics about the structural conformation of β -s protein.

Next step in our work its to use MD to analyze the movility of α -s and β -s protein in order to dilucidate the influence of the secondary structures on the stability of this odd behaving protein, and to create pH physiologically correct representations.

Conclusions

The Dr. Jekyll and Mr. Hyde methapora, represents the dual character of this protein. The ability to participate in different type of processes must be understood if we want to find effective alternatives for the aggregation inhibition process of α -s.

Our aim is to understand, within the framework of computational chemistry, the influence of β -s protein aggregation inhibition of α -s protein. This work is a first approximation in order to understand the influence of the structure of this proteins, from a view point of molecular mechanics.

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