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Impact of macromolecular crowding on structure and properties of pepsin and trypsin

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ABSTRACT

The change of conformation of pepsin and trypsin in absence and presence of a high molecular crowding agent has been characterized using dynamic light scattering (DLS). Structural properties were investigated as a function of chemical denaturant concentrations, the guanidine hydrochloride (GdmCl). The results showed that Ficoll 400, macromolecular crowding, has a strong effect on the chemical denaturation process of these two proteins. The changes of measured hydrodynamic radius are attributed to the enhancement effect of the crowder agent due to the excluded volume effects. The data proved that the large size of a macromolecular crowder plays a crucial role on the conformation of a protein in its unfolded states. The values of interactions Parameter k_d of complex particles and a number of proteins n_{pr} attached on the Ficoll 400 measured in different GdmCl concentrations. The effect of aging on the structure of complex are studied by small angle light scattering (SALS).

1. Introduction

The growth and improvement of our knowledge of biophysical properties of proteins in native and denatured states have gained an outstanding attention to its importance for the understanding of their function in nature. The interest of these polypeptides aroused since their applications in many fields like the food industry, cosmetics, medicine and biological fields due to their unique folded structure. These proteins express specific functional properties like enzyme catalysis, ligand binding and transport of biologically active molecules, and also water solubility, flavor binding, gelation, emulsification and foaming properties. Biological functions of proteins, in general, require correct protein folding and protein stability. However, folding of a protein is an important process to fulfill their specific function. Several studies on unfolding and refolding of chemically and thermally denaturation process of proteins have guided a deep understanding of protein folding. But the conditions of those studies were different from the cell environment. Hence, the presence of soluble and insoluble macromolecules makes the cell interior densely crowded [1–3]. However, the crowded environment was always related and explained by the excluded volume theory [2,4]. For this purpose, the stability of protein under a molecular crowding agent has been widely studied [5–7]. Many authors have studied the effect of molecular crowding on the stability of native and denatured states of proteins and have shown that the excluded volume has a major effect on the affinity of proteins [8–13]. Indeed, the excluded volume theory, based on the result of De kruif, predicts the depletion forces [14], which they are formed between a large molecule and small molecules suspended in solution, i.e. the large particle excluded the small particles from their proximity. The depletion forces were known as the effective attractions between two spheres in a crowded solution [14].

Our aim is to know, the role and effects of crowder agent, with a large size, on the conformation of unfolded proteins and to better understanding the key parameters implied in protein–polysaccharide interactions. We examined, under the presence of Ficoll 400, the denaturation of pepsin and trypsin separately by the guanidine hydrochloride (GdmCl).

Dynamic light scattering (DLS) is known to be a very powerful tool that yields low and medium resolution information about the structure of macromolecules in solution. This technique has been applied to inform us on the changes of the molecular size of proteins in the presence or not of a molecular crowding agent [15–19]. This technique provides a direct measurement of the hydrodynamic radius of molecules, and it is very sensitive to the compactness of molecules which is a strong enough parameter to characterize the degree of denaturation polypeptides. This work is a prolongation of obtained results by Aschi et al. [15], which followed the conformational changes for protein during the chemical and thermal denaturation by DLS, but in the presence of crowding agent, the Ficoll 400. Still, in this work, we demonstrated that ficoll dramatically enhanced the denaturation of these proteins, pepsin and

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trypsin. Our results suggest that the size of the crowder agent has a significant importance on the conformation of proteins.

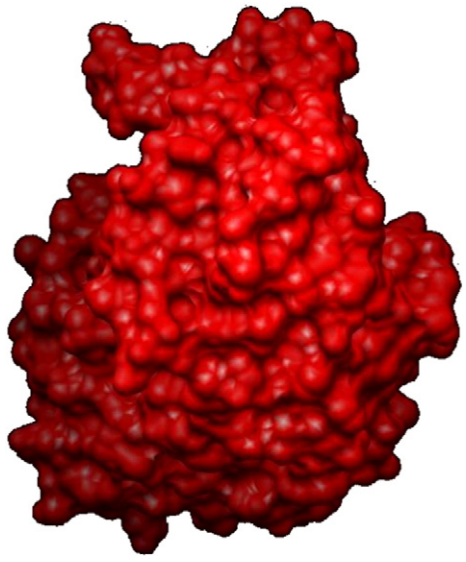
The effect of aging on the structure of the Ficoll 400/pepsin mixture are studied by small angle static light scattering (SALS). This technique allows us to determine the radius of gyration and fractal dimension of studied macromolecules in solution.

2. Materials and methods

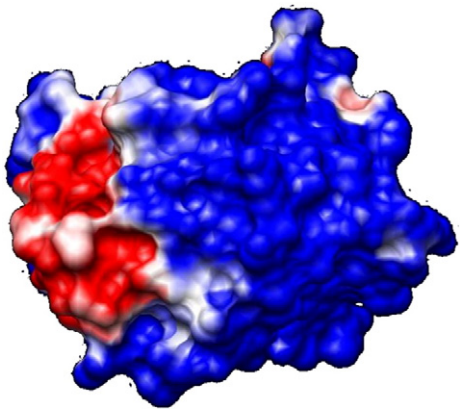
2.1. Materials

Pepsin was purchased from Prolabo (molecular weight = 34.5 kDa). Trypsin was purchased from Fluka Biochemika (N°93613) (molecular weight = 23.5 kDa). Ficoll 400 (molecular weight = 400 kDa), guanidine hydrochloride (GdmCl) was the ultrapure sample from Sigma Chemical Co. Protein solutions were dialyzed extensively against 0.1 M KCl at pH 7.0 and 4 °C, and the stock solution was filtered using 0.22 mm Millipore syringe filter. All samples were dissolved in 0.1 M phosphate buffer, containing 0.1 M NaCl, in order to reduce the electrostatic interactions. The pH of the solution was adjusted to 7. The protein concentration was determined by absorbance at $\lambda_{max} = 280$ nm ($E^{1\%} = 14.7$ for pepsin and $E^{1\%} = 12.9$ for trypsin). The molar concentration of guanidine hydrochloride [GdmCl] was determined by the refractive index measurements.

Pepsin (Scheme 1) is a globular protein and it is widely used such in pharmaceutical therapeutics for the treatment of some gastrointestinal diseases. Trypsin is medium-sized globular protein (Scheme 2) also used in pharmaceutical therapeutics as an anti-inflammatory agent. Pepsin and trypsin are enzymes produced in the stomach of humans or animals. They serve to decompose the proteins contained in the food into peptides. These enzymes exist with another principal protease in the digestive system. The choice of these proteins was based on the fact that the optimal pH for the enzymes pepsin (pH = 2) and trypsin (pH = 8) is different. The behavior of pepsin enzyme is different from that of other enzymes such as, trypsin, papain, ribonuclease and chymotrypsin. Indeed, in the case of pepsin, a high concentration of chemical denaturant (urea or guanidine hydrochloride) causes a weak effect on the alteration of secondary structure and his catalytic activity is weakly impaired. In addition, the folding of Pepsin enzyme is sensibly different from that of the other enzymes cited above and the biological activity is not affected [20]. Au contrary, a significate effect on the above mentioned properties has been observed for trypsin.



Scheme 1. Electrostatic potential contour (+1 kT/e (blue) and −1 kT/e (red)) around the pepsin at ionic strength 0 M. pH value and corresponding net charges: 7.0, ~−37.9 calculation was based on PDB ID 5PEP.



Scheme 2. Electrostatic potential contour (+1 kT/e (blue) and −1 kT/e (red)) around the Trypsin at ionic strength 0 M. pH value and corresponding net charges: 7.0, ~+6.3 calculation was based on PDB ID 2PTN.

The crowder agent is Ficoll 400. Ficoll 400 is a highly internally-crosslinked copolymer of sucrose and epichlorohydrin. The high content of hydroxyl groups makes this macromolecule soluble in aqueous media and behaves as a compact globular particle in solution (ideal neutral sphere) [21,22]. Furthermore, Ficoll 400 is non-charged inert macromolecules that have been used, in several studies, as macromolecular crowding for proteins [23,24].

2.2. Dynamic light scattering

Dynamic light scattering (DLS) measurements were performed at fixed protein concentrations of 2 mg/ml and temperature fixed to 25 °C. All experiments were performed at 90° scattering angle and at a wavelength of 633 nm. DLS measurements have been performed in the so-called parallel mode, which allows measurements of the intensity-intensity autocorrelation function $g^{(2)}(t)$ over a wide time logarithmic scale. In this mode the measured relaxation time, τ can be probed.

The intensity-intensity autocorrelation function $g^{(2)}(t)$ is related to the electric field autocorrelation function $g^{(1)}(t)$ by the following Siegert relationship:

$$g^{(2)}(t) = 1 + \beta\left|g^{(1)}(t)\right|^2 \quad (1)$$

where $0 < \beta < 1$ is dependent on experimental values (e.g. detection optics).

Consider the case for a dilute solution, the function $g^{(1)}(t)$ is simply equal to the following relationship:

$$g^{(1)}(t) = A_{slow}\, e^{-t/\tau slow} + A_{fast}\, e^{-t/\tau fast} \quad (2)$$

where A_{slow} and A_{fast} are the amplitudes of the slow and the fast modes, respectively, and τ_{slow} and τ_{fast} are the corresponding relaxation times.

The fast relaxation time τ_{fast} is related to the apparent diffusion coefficient D_{app} by the following relationship:

$$D_{app} = 1/(q^2\tau_{fast}) \quad (3)$$

The scattering wave vector q is related to refractive index n in the relation, $q = \frac{4\pi n}{\lambda}\sin(\frac{\theta}{2})$, where θ is the scattering angle and λ is wave length. We measured the apparent diffusion coefficient D_{app} for different concentrations in the chosen range.