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Self-assembling of poly(aspartic acid) with bovine serum albumin in aqueous solutions

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ABSTRACT

Macromolecular co-assemblies built up in aqueous solutions, by using a linear polypeptide, poly(aspartic acid) (PAS), and a globular protein, bovine serum albumin (BSA), have been studied. The main interest was to identify the optimum conditions for an interpenetrated complex formation in order to design materials suitable for biomedical applications, such as drug delivery systems. BSA surface possesses several amino- and carboxylic groups available for covalent modification, and/or bioactive substances attachment. In the present study, mixtures between PAS and BSA were investigated at 37 °C in dilute aqueous solution by viscometry, dynamic light scattering and zeta potential determination, as well as in solid state by AFM microscopy and dielectric spectroscopy. The experimental data have shown that the interpolymer complex formation occurs for a PAS/BSA molar ratio around 0.541.

1. Introduction

During the recent decades, considerable attention has been devoted to polymer–polymer interactions and interpolymer complexes formation. This interest is motivated by a number of fundamental and application-oriented problems, being of significant importance because of the possibilities for designing and fabrication of novel polymeric co-assemblies, structures, and materials. Such macromolecular co-assemblies (also referred as interpolymer complexes) possess unique and remarkable properties that are different from the properties of their polymeric components [1]. A particular attention is focused on self-organization phenomena in macromolecular systems based on synthetic polyelectrolytes and natural macromolecules. In this context, the complexation of proteins with natural and synthetic polyelectrolytes is of interest at least from two points of view. The first one concerns the way in which the polymers interact with nonflexible protein molecules, which could provide a better explanation of the interaction mechanism of polyelectrolytes with ionic colloidal particles. The second one refers to the biochemical activity of protein in the resulting protein-polymer system, this behaviour being of importance for biomedical applications of the complexes [2]. The main conclusions derived from previous studies [2–8] may be summarized as follows: (i) protein-polyelectrolyte complexes (PPCs) are formed mainly through electrostatic forces; (ii) in salt-free systems, at least, protein molecules are complexed with flexible polyelectrolytes through the stoichiometric formation of ion pairs (or salt linkages) between oppositely charged groups; (iii) the ion pairs between the polyelectrolyte and protein molecules are very labile and may be severed by changes in pH as well as by the addition of small ions or polyions; (iv) there is an appreciable retention of biochemical function in the resultant complexes.

Both natural and synthetic polyelectrolytes form strong complexes with a variety of proteins [9–13]. Protein-polyelectrolyte complexes resulting from coupling oppositely charged (or getting charged) polyelectrolytes represent an important and significant part in the domain of self-organizing polymer systems. Molecular self-assembly is based on spontaneous organization through non-covalent interactions of the molecules, under near thermodynamic equilibrium conditions, into structurally well-defined and stable arrangements. Such interactions typically include hydrogen bonding, electrostatic attraction and van der Waals interactions. Self-assembling peptides have received a great deal of attention for researchers in biomaterials and nanoscience field since these products can be engineered to form a wide variety of nanostructures and hold considerable promise for a broad range of applications [14].

In the present paper, macromolecular co-assemblies built from poly(aspartic acid) (PAS), a linear polypeptide, and bovine serum albumin (BSA), a globular protein, have been studied. PAS, which belongs to the synthetic polypeptides family, is a biocompatible

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and biodegradable water-soluble polymer. Due in part to the carboxylic groups, PAS has some similarities in chemical properties with poly(acrylic acid) [15] and forms interpolymer complexes with other synthetic polymers [16–19]. On the other side, bovine serum albumin (from cows and known as BSA or "Fraction V") is a monomeric globular protein. BSA has relatively high molecular weight, 17 disulphide bonds, highly flexible free cysteine (Cys34), and slightly lower pKa value (5–7) compared to other cysteines (8–11) [20,21]. This new system composed of PAS and BSA was selected for the present study on the basis of the following considerations: (i) the main interest was to obtain an interpenetrated complex suitable for bioapplications; (ii) the surface of the new complex was expected to possess amino- and carboxylic groups derived from both partners, which would be available for further modifications and covalent attachment of bioactive substances [22]; (iii) in recent years, considerable interest has been shown for the use of natural proteins as a carrier for drug delivery [23,24].

The albumin carriers are suitable for drug delivery since they are biodegradable, biocompatible and relatively easy to prepare over a wide range of particle sizes [25]. In a recent paper [17], the evaluation of the PPC formation from PAS and BSA at different temperatures was made by using different techniques such as: spectroscopy, microscopy, thermogravimetric behaviour, DLS and zeta potential analysis. The trend of the hydrodynamic diameter, zeta potential and conductivity, as function of temperature, evidenced the interactions intervened between partners. Thus, a more relaxed structure of PPC was obtained at 37 °C. As result, this temperature was chosen for the present investigation, which is of interest for biomedical applications. In this study, the properties of BSA were combined in a synergistic manner with the amphiphilicity and surfactant properties of PAS. The conditions for the complex formation between BSA and PAS were examined. In this context, the specific techniques such as viscometry, DLS analysis, microscopy and dielectric spectroscopy were used for characterization and evaluation of the PPC formation between PAS and BSA.

2. Experimental

2.1. Materials

Poly(aspartic acid) (PAS) was prepared as it was described previously in detail [26]. The synthesis is based also on a reaction in two steps: in the first step, the precursor poly(succinimide) (PSI) was prepared by thermal polycondensation in mesitylene/sulfolane (Fluka Chemika provenience) of *L*-aspartic acid (Fluka BioChemika provenience), at 180 °C, for 6 h, with *o*-phosphoric acid (analytical reagent of 85%, Chemical Co. provenience) as catalyst; but in the second step, PSI was hydrolysed in alkaline medium at −5 °C for 1 h. According to this procedure, the obtained PAS sample had an increased molecular weight: M_n = 25,790 g/mol, M_w = 31,820 g/mol, M_w/M_n = 1.234 (from GPC data).

A sample of bovine serum albumin (BSA) provided by Sigma (A-7906 Lot 30K0932, minim 98% electrophoresis, Fraction V), as a variety of bovine serum albumin with purity of 95–99% and molecular weight of ~66,000 g/mol, was used as globular protein.

2.2. Samples preparation

Aqueous solutions of PAS and BSA, with a concentration of 1 g/dL, were prepared by dissolution of each sample into Millipore water under magnetically stirring for 60 min. The homogeneous solutions were kept at rest in refrigerator at 5 °C for 24 h. The PAS/BSA mixture was prepared by direct mixing of various ratios of the PAS and BSA solutions and stirred for 60 min. For all samples, PAS was added into the BSA solution in order to avoid differences in behaviour during the experiments. Further, the following obtained PAS/BSA molar ratios, x^*, respectively: 0.000, 0.116, 0.283, 0.541, 0.691, 0.780, 0.861, 1.000 were investigated. The samples were prepared at pH 6.4 (adjusted by means of HCl or NaOH by using the Autotitrator Malvern MPT2 device).

2.3. Viscosity measurements

The aqueous solutions of PAS, BSA and their mixtures were investigated in dilute solution at 37 °C by using an Ubbelohde capillary viscometer (type 0a with a capillary diameter of 0.53 mm) in combination with an automatic viscosity measurement system (Lauda Instrument, Germany). Solutions with different polymer concentrations were prepared by sequential dilution of initial stock solutions, directly inside the viscometer. Upon dilution, prior the measurements, each solution was kept about 15 min for thermal equilibration. Flow times were obtained with good reproducibility and the errors were less than 1%. All samples were free of dust by means of filters with a pore diameter of 0.45 μm. Only freshly prepared samples were used. The initial solutions for viscosities studies were prepared at pH 6.4 (adjusted by means of HCl or NaOH by using the Autotitrator Malvern MPT2 device). Further dilutions were made with Millipore water (pH = 6). After dilution, the pH was not monitored.

2.4. DLS analysis

2.4.1. Particle size determination

The measurements of the particle size were performed by *dynamic light scattering technique* (Nano ZS Zetasizer model, Malvern Instruments, UK), with a red laser wavelength of 633 nm (He/Ne). The system uses a non-invasive back scatter (NIBS) technology (which reduces the multiple scattering effects) wherein the optics is not in contact with the sample, back scattered light being detected [26,27]. On the whole measuring range from 0.6 nm to 6 μm, the system applies the Mie method. Dynamic light scattering (DLS) measurements yield the Z-average of the aggregate's apparent hydrodynamic radius (R_H), according to the following equation:

$$R_H = \frac{kT}{6\pi\eta D} \quad (1)$$

where R_H is the hydrodynamic diameter, k the Boltzmann constant, T the temperature, η the viscosity and D is the diffusion coefficient. The hydrodynamic diameter, often expressed by symbol Z or z-average, is calculated from signal intensity. Solutions with concentrations of 1 g/dL and different molar ratios between PAS and BSA (x^*) were investigated.

2.4.2. Zeta potential evaluation

Zeta Potential (ζ) was determined on the same Zetasizer Nano ZS device by using Smoluchowski relationship:

$$\zeta = \frac{\eta\mu}{\varepsilon}, \quad \text{and} \quad k\alpha \gg 1 \quad (2)$$

where: μ is the electrophoretic mobility, η – viscosity, ε – dielectric constant, k and α – Debye-Hűckel parameter and particle radius. Also, the *electric conductivity* was determined concomitantly with zeta potential measurements. The analyses were performed at pH 7.4 (adjusted by means of HCl or NaOH done by with the Autotitrator Malvern MPT2 device) and at 37 °C (temperature maintained constant at (±0.1 °C) with a Peltier device). Each measurement was made 3 times and the average values were considered.