Amphiphilic poly-N-vinylpyrrolidone nano-carriers for protein encapsulation

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Introduction

As a consequence of the great advances in biotechnology during the last decade therapeutically relevant peptides and proteins have been a focus of considerable interest. Although they are very potent drugs they possess a number of troublesome intrinsic properties such as their short plasma half-life and poor solubility. Drug delivery systems can help to overcome these problems. Encapsulation of peptides and proteins into biodegradable polymers protects them against enzymatic inactivation and their release can be controlled to maintain therapeutic plasma levels.

In the past few years, there has been an increasing interest in developing stealth nanoparticles as drug or protein carrier systems to avoid rapid drug clearance from the systemic circulation. One of main methods for preparation of stealth or long-circulating nanoparticles is to modify their surface with a hydrophilic, flexible and nonionic polymer, such as, for example, poly(ethylene glycol) (PEG) (M. Tobyo et al., 1998). The stealth nanoparticles compared to other long-circulating systems show better shelf stability and ability to control the release of the encapsulated compounds, but information on the stealth nanoparticles used to deliver protein drugs has not been clear so far.

The aim of the present work was to assess the merits of nanoparticles on the basis of another amphiphilic non-ionic polymer, poly-*N*-vinylpyrrolidone (PVP), as a protein drug carrier. For this purpose, amphiphilic PVPs of molecular weight (MW) 2600, 4000 and 6500 with one end hydrophobic octadecyl group (PVP-OD) were synthesized using previously developed methods (A.N. Kuskov et al., 2005). Bowman–Birk soybean proteinase inhibitor (BBI) and its hydrophobized oleic acid derivatives (Ole_1-BBI and Ole_2-BBI) were chosen as model proteins. BBI can be used for simultaneous inhibition of trypsin and chymotrypsin. Also, BBI actively suppresses cell transformation *in vitro* and carcinogenesis *in vivo* (W. Troll et al., 1993). Still, the solubility of BBI and its hydrophobized derivatives is very low, limiting their application in medicine. All the BBI preparations were encapsulated within nanoparticles made of PVP-OD. The particles were characterized in terms of size, shape, *in vitro* release of the protein and antitryptic activity.

Materials and Methods

N-vinylpyrrolidone, Bowman-Birk soybean proteinase inhibitor and other reagents were purchased from Sigma, USA; trypsin and chymotrypsin were purchased from Merck, Germany. All solvents and components of buffer solutions were analytical grade preparations.

Amphiphilic poly-N-vinylpyrrolidones of different molecular weight hydrophobically α-end-capped with octadecyl groups (PVP-OD) were synthesized in two steps, similar to our previous experiments (A.N. Kuskov et al., 2005).

The BBI, modified by one (Ole_1-BBI) or two (Ole_2-BBI) oleic acid residues, was prepared using previously developed methods (E.V. Malykh et al., 2002). The investigation of amphiphilic PVP self-assembling processes in absence and in presence of BBI preparations was carried out by

addition of appropriate amounts of protein to the PVP-OD solutions with different concentrations. The mixture was sonicated using a Sonic Desmembrator 60 (Fisher Scientific, USA). For protein isolation from colloid system it was precipitated by acetone. The sediment was centrifuged and dried out. The purity of protein was controlled electrophoretically.

To estimate the critical aggregation concentration (CAC) values for different PVP derivatives, the method used was based on the solubilization of water-insoluble fluorescent dye, pyrene, in nano-aggregates. The fluorescence intensity of solubilized pyrene was measured using a Hitachi 650-10 S Spectrophotometer (Japan). The average size of polymeric nano-aggregates obtained in water and in physiological solution and their size distribution were determined by dynamic light-scattering (Malvern S4700 PCS System, UK) The form of amphiphilic PVP aggregates was determined by transmission electron microscopy (TEM) using apparatus JEOL JEM-1000 (Germany).

The antitryptic activity of BBI preparations was determined by relative esterase activity of trypsin stayed free after its incubation with inhibitor samples. The changes of the optical density were registered at a wavelength of 253 nm with spectrophotometer Shimadzu UV-265 FW (Japan).

For protein release kinetics study the suspension of polymeric nanoparticles with included proteins was resuspended in phosphate (pH 7,4) or acetate (pH 5,5) buffer. Obtained suspension was incubated at 37 ºC, and the samples were taken after 1, 6, 12, 24, 48, 60, 96 and 120 hours. The samples with added NaCl were centrifuged using ultra centrifuge Beckman Coulter (Germany). The supernatant was isolated and protein concentration was determined using Lowry method with BBI solution as a standard. The antitryptic activity of released protein was determined against tripsin.

Results and Discussion

Since synthesized polymers have the amphiphilic nature, the polymeric nanoparticles of amphiphilic poly-N-vinylpyrrolidones were prepared in aqueous media. Using the analysis of fluorescence excitation spectra and dynamic light-scattering it was shown that ability to form nanoparticles of 30-700 nm size for polymers with primary octadecyl end group increases with decreasing the polymer molecular weight. In all cases the size of aggregates formed in physiological solution is lower than for those which were obtained in distilled water.

The electrophoresis of BBI and PVP-OD mixture revealed the presence of protein band with higher electrophoretic mobility than for native protein. This effect confirms the presence of BBI combined with PVP-OD. The results of dynamic light-scattering investigations for different BBI derivatives and PVP-OD mixtures indicate that the increasing of BBI preparations concentration in solution leads to the decreasing of particles size. Probably, the presence of protein compacts and regulates the structure of colloid aggregates. It should be mentioned that in contrast to the native BBI, Ole_1–BBI and Ole_2–BBI initiate formation of complex aggregates at lower concentrations of PVP-OD. Both of the hydrophobized BBI derivatives decreased the average size of forming particles and constricted their size distribution (Figure 1). This difference can be explained by the increased affinity of protein to amphiphilic PVP-OD due to strengthening of hydrophobic interactions between them after attaching of one or two oleoylic residues. Using the transmission electron microscopy it was shown that obtained particles are of spherical form (Figure 2).

Also it should be emphasized that at certain ratio of PVP-OD and BBI preparations concentrations in the mixture, the protein becomes fully dissoluble in aqueous medium. The average size of the particles decreases even to 50 nm.

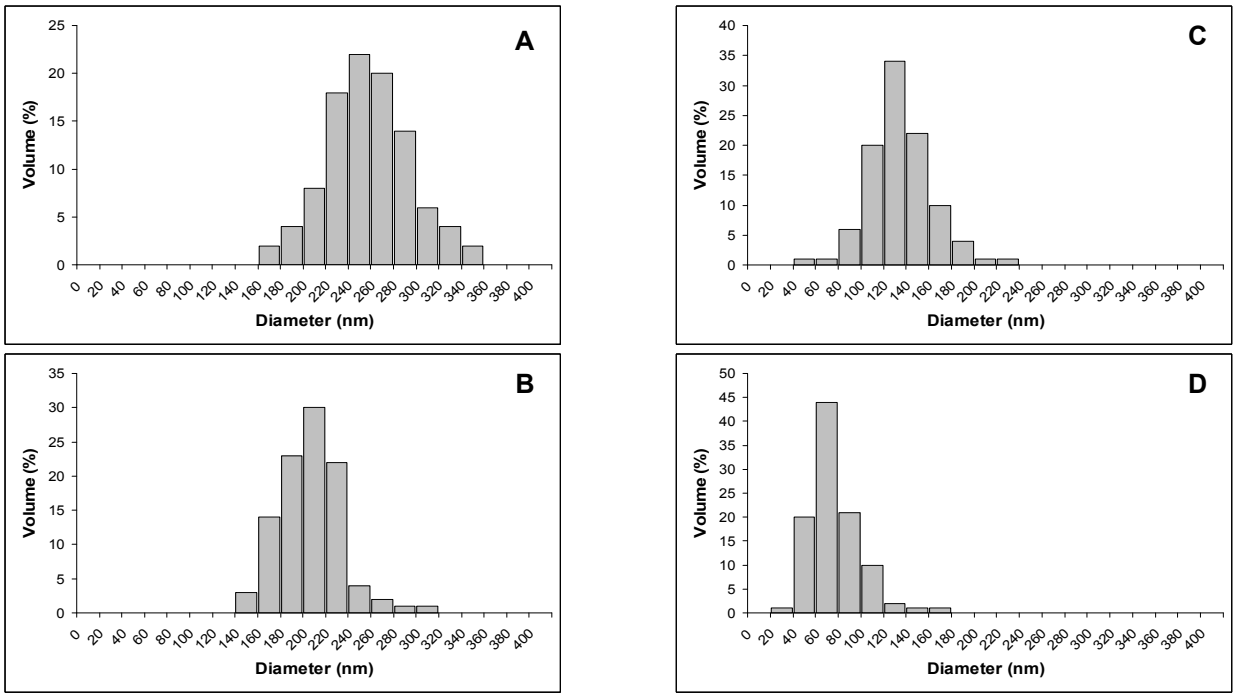

Figure 1: Size distribution of PVP-OD 4000 particles in physiological solution. A – PVP-OD 4000 (0,5 mg/ml); B – PVP-OD 4000 (0,5mg/ml) + BBI (2,0mg/ml); C – PVP-OD 4000 (0,5 mg/ml) + Ole$_1$–BBI (2,0 mg/ml); D – PVP-OD 4000 (0,5mg/ml) + Ole$_2$–BBI (2,0mg/ml).

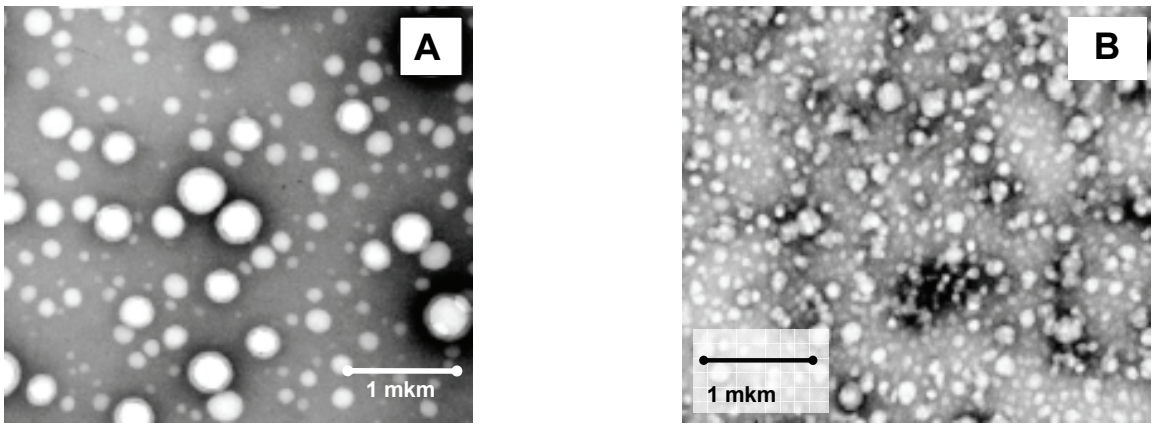

Figure 2: Prepared polymeric nano-particles photomicrography obtained by TEM method. A – PVP-OD 2600 (C=0,5 mg/ml); B – PVP-OD 2600 (0,5 mg/ml) + Ole$_2$–BBI (2,0 mg/ml).

It is well-known that during the solubilization process the substance is included in the particle core and its interaction with environment is impossible. Denaturing effect of solution with low pH value (1.4) was measured against plain Ole$_2$–BBI preparation in aqueous medium and against Ole$_2$–BBI which was encapsulated in polymeric aggregates. As one can see on the Figure 3, I, plain Ole$_2$–BBI preparations loose 80 % of their antitryptic activity in 24 hours and are fully inactivated in 48 hours. Instead of this, the protein encapsulation in polymeric aggregates leads to the preservation of its activity (Figure 3, II). In the mixture of amphiphilic polymer with protein consisting basically of large aggregates, protein activity lowers by 60 % in 48 hours. It can be assumed that in this case protein not included in particles core and localized between polymer chains of large aggregates, can interact with water medium and becomes inactivated. At the same time formation of small micelle-like polymeric particles and encapsulation of protein in these particles lead not only to increasing of preparation solubility but to practically total preserving of inhibitor activity (90 %) (Figure 3, III).

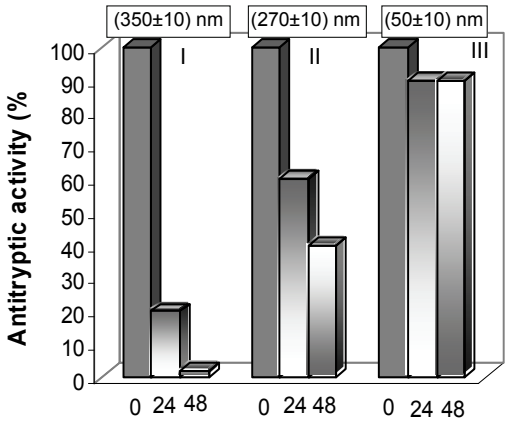

Figure 3: BBI antitryptic activity: I - Ole_2–BBI (0,5mg/ml); II - PVP-OD 6500 (0,5 mg/ml) + Ole_2–BBI (0,5mg/ml); III - PVP-OD6500 (0,5mg/ml)+Ole_2–BBI (2,0 mg/ml).

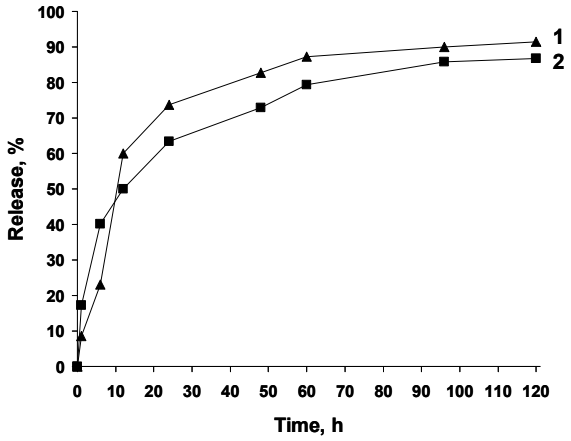

Figure 4: BBI release from PVP-OD2600 nanoparticles in 0,05 M phosphate (pH 7,4; 37ºC) (1) or acetate (pH 5,5; 37ºC) (2) buffer.

Another problem of protein immobilization using polymers is that this processes lead to irreversible conformation changes in structure of protein globule and to full loss of protein activity. In this work the BBI release kinetics from the polymeric nanoparticles on the basis amphiphilic PVP derivatives was studied and the biological activity of released protein was determined. The BBI release kinetics from PVP-OD 2600 nanoparticles (Figure 4) shows that after 48 h of incubation in phosphate (pH 7.4) or acetate (pH 5.5) buffer practically complete protein release is observed. The BBI activity against trypsin was determined after 48 h incubation of polymeric nanoparticles. The activity was about 85–90%. This result points to the stability of the protein to the processes of encapsulation.

Conclusions

Polymeric aggregates of PVP-OD with and without encapsulated native or hydrophobized proteinase inhibitor (BBI) were prepared and characterized. It was shown that addition of proteins to PVP-OD aqueous solutions can provide formation of small micelle-like particles (50 nm) fully solubilizing protein (increasing water-compatibility) and preventing it from inactivation. Obtained results allow considering novel polymers as promising basis for protein drug encapsulation

References

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