Interactions between protein and stereoregular polyelectrolyte

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Introduction

Investigations of protein-polyelectrolyte complexes are important in the design of many biomedical applications that include enzyme immobilization, drug delivery, biosensor design, and in bioprocessing where complexation with polyelectrolytes is used to prevent protein aggregation and for protein purification. In this work, we choose to study the effect of polyelectrolyte stereochemistry on the interactions between sulfonated polystyrene (PSS) and myoglobin. We have sulfonated polystyrene with a unique stereochemical structure or tacticity, i.e (a) atactic polystyrene (aPS), and (b) isotactic polystyrene (iPS). aPS is a typical amorphous polymer and the stereochemistry of atactic polystyrene shows some long range syndiotacticity. iPS is a crystalline polymer and in solid state, it has a conformation of 3-1 helix while in gel state it is more stretched and has a helix of 12-1 conformation. The sulfonated states of aPS and iPS, ie, aPSS and iPSS are soluble in water with special local conformation. In this paper, we will report the different binding strength of PSS with myoglobin.

Experiment Section

Materials and solution preparation

Isotactic polystyrene was synthesized by the method of Natta and low polydispersity sample was obtained by the toluene-methanol method. Sulfonation was carried out by introducing

concentrated sulfuric acid. Sodium salt was obtained by adding NaOH. Atactic polystyrene sulfonate sodium was ordered from Aldrich. Horse heart myoglobin was ordered from Sigma and to eliminate excess salts and impurities, myoglobin was dialyzed against Ph7,Tris-HCl buffer with 10mM Nacl. New portion of buffer was changed every 8 hours and the last portion was kept for further use. Both apss and ipss were dissolved in the last portion of buffer to equilibrate the pH and ionic strength of the solutions.

UV-vis Spectrometry

Absorbance spectra were measured using a Beckman Spectrophotometer with quartz cuvettes, operating in the UV-visible region, with a full scale expansion of 0.2 absorbance units.

Results and Discussion

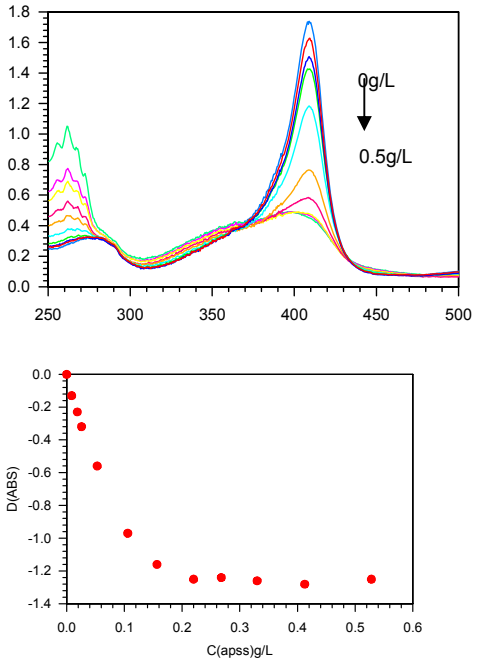

Figure1.Apss binding to myoglobin at PH7 10mM Nacl

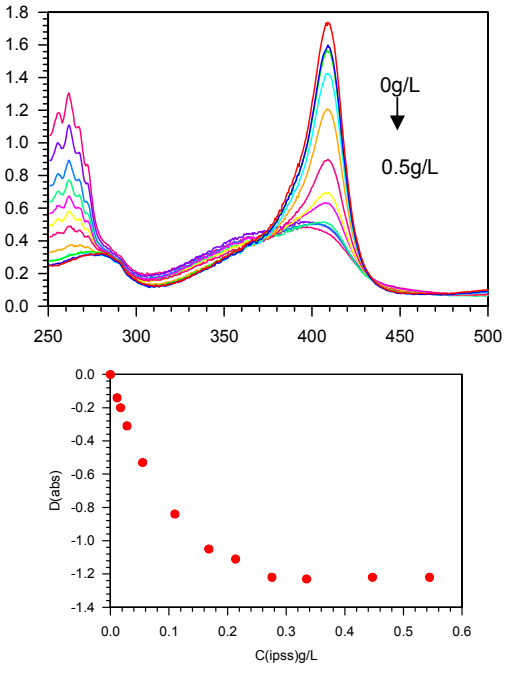

Figure2 Ipss binding to myoglobin at PH7 10mM Nacl

Figure 1 and Figure 2 shows the binding isotherm of myoglobin to apss and ipss, respectively. The

binding shows a clear decrease in the 409nm absorbance band which means that the binding leads to the denaturation of myoglobin. The denauration process of myoglobin can be treated as a two state process and in our case, we can treat the process as native and bounded states. So the fraction of bounded myoglobin can be obtained by the equation1.

$$F_B = \frac{A_N - A_{OBS}}{A_N - A_B} \quad (1)$$

Where where A_{OBS} is the absorbance observed and A_N and A_B are the absorbances for the native and bounded myoglobin, respectively.

In our experiment, myoglobin could occupy more than one binding site on the PSS chain had to be considered so as to prevent any overestimation of the potential binding sites on the PSS. We employed the binding model of McGhee and von Hippel[1] in which complexation between ligand and macromolecule, can be expressed with the equation 2.

$$\frac{v}{L} = K(1 - nv)\left[\frac{1 - nv}{1 - (n-1)v}\right]^{n-1} \quad (2)$$

Where L is the free ligand (myoglobin) concentration (M), K is the binding constant (M-1), n is the average size of the binding site (number of PSS monomers covered with a myoglobin monomer), ν is the binding density (number of myoglobin complexed per PSS monomer).

With the least square fitting of the UV data, the binding results are summarized in Table1.

Table1. Binding results of PSS to myoglobin

sample	Binding constant K(M-1)	Binding site
aPSS+Myoglobin ph7 10mM NaCl	5.48E4±1.37E4	35±3
iPSS +Myoglobin ph7 10mM NaCl	1.55E4±0.17E4	33±2

From the binding results, we can see that the binding of myoglobin with apss is stronger than the binding with ipss. Vlachy[2] et al modeled the isotactic chain and atactic chain of poly(methacrylic acid) with quantum mechanics method. And showed that the isotactic chain in solution tend to form both helical structure and bent structure while atactic chain tends to form bent structure with larger torsional angles than isotactic bent conformation. This results in a higher chain flexibility of atactic chain than isotactic chain in solution. And the higher chain flexibility of apss leads to more efficient ion pairing than that of ipss.

Conclusion

Our results show that although the macroscopic solution behaviors are quite the same for ipss and apss. The binding of apss with myoglobin is stronger than ipss due the higher local chain flexibility of apss. The results show that the binding of polyelectrolyte to protein is a quite local process so detailed structure analysis needs to be considered.

Reference

1. James D. Mcghee and Peter H. Von Hippel, Journal of Molecular Biology ,1974, 86, 469-489
2. Nina Vlachy, Jozica Dolenc, Bostijan Jerman and Ksenija Kogej, Journal of Physical Chemistry,B 2006,110,9061-9071