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Influence of pH and temperature on the structure in macromolecular solutions. A preliminary SANS study on lysozyme

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ABSTRACT

In this paper we present some preliminary Small Angle Neutron Scattering results about the influence of pH and temperature on the structure of macromolecular solutions. We have investigated aqueous solutions of lysozyme at concentrations of 0.05, 0.10 and 0.15 w/w, at pH values of about 3, 5, and 6.7, and in the temperature range of 10–50°C. At the lower values of the pH, well defined interaction peaks are present in the scattered intensities, whereas at the higher pH an unstructured aggregation takes place, which is reduced by the increase in temperature.

1 Introduction

Biological macromolecules consist in linear sequences of aminoacids wrapped in a well defined three dimensional shape. The inner part of macromolecules is composed of hydrophobic residues of the aminoacids, whereas the outer part is mainly composed by the polar residues. When macromolecules are in water they behave as macroions, with a well defined distribution of charges on their surfaces. For a protein with a given isoelectric point, the charge distribution is controlled by the pH of the solution. The interaction among macromolecules in solution, basically arise from the net charge and from the dipoles and multipoles induced by the charges. The interaction potential is therefore driven by a competition between an attractive part generated by dipolar and dispersive forces, and a repulsive part caused by the coulombian contribution.

We are interested in macromolecular solutions since we have observed that in particular chemical-physic conditions these solutions show peculiar rheological properties that were ascribed, by light scattering measurements, to the formation, on a mesoscopic scale, of structured cluster of macromolecules(1,2). More recently SANS measurements revealed that on a smaller scale the macromolecules are organized in the solutions, and form structured aggregate(3,4). The type of structure that is formed by the macromolecules should depend on the pH of the solution. At a pH close to the isoelectric point the net charge is nearly zero so that only the attractive part of the potential is present and flocculation of the macromolecules occurs. Very far from the isoelectric point only the repulsive part of the interaction is present, and the macromolecules should be spaced apart at the maximum distance allowed by the concentration. At intermediate values of the pH the competition between the two terms may give rise to well defined structures that account for the observed unusual properties. To a better understanding of the role played by the attractive and repulsive part of the potential we have performed SANS measurements on aqueous solutions of Lysozyme at different pH; the temperature dependence of the scattered intensity has also been investigated. In this paper we present a preliminary analysis of the collected data.

2 Experimental

Lysozyme is a globular protein with a molecular weight equal to 14000 dalton, with the shape of a prolate ellipsoid ($27 \times 44 \text{ \AA}$). The isoelectric point of this enzyme is at $\text{pH}=11$. The sample has been purchased from Sigma Chem. Co (three times crystallized). The solutions were prepared at the concentrations of 0.05, 0.10 and 0.15 (grams of solute/grams of solution) in heavy water. We prepared solutions at $\text{pH}=3$ using a Na-citrate-HCl buffer, and at $\text{pH}=5$ and $\text{pH}=6.7$ using a phosphate buffer. For the solution at $\text{pH}=3$ and 6.7 we also investigated the temperature dependence in the range of $10\text{--}50^\circ\text{C}$. To collect the SANS spectra we used the PAXE spectrometer at the ORPHEE reactor (Saclay), and standard procedures have been used to obtain absolute units.

3 Discussion

The scattered intensity recorded for the three studied concentrations—0.05, 0.10, 0.15—are shown in figures 1(a, b, and c). In each figure the different pH that we used—3, 5, and 6.7—are shown with the same symbol. The general behaviour of the scattered intensity at the various pH, as observed in the figure, is similar for the three concentrations. At $\text{pH}=3$ and $\text{pH}=5$, a well defined interaction peak is present which, at the lower pH value, appears to be broader and slightly shifted toward smaller wave-vectors. The presence of the interaction peaks reflects the existence of some kind of structure among the macromolecules in the solution.

The case $\text{pH}=4$ has been analyzed in a more detailed way in a previous paper(3). In fact in that case we have extracted, for each concentration, the Structure Factors that were analyzed in terms of a *preferred distance* between the macromolecules and a *mean square displacement* around that distance. The preferred distances we have found are, at each concentration, systematically smaller than a *mean distance* ($n^{-1/3}$) obtained from the specific volume available for a macromolecule at that values of the concentration number n . This circumstance has been interpreted as the effect of structured clusters of macromolecules in the solution, due to the particular minimum in the interaction potential generated by the competition of the attractive

and repulsive part of the interaction.

The fact that at a pH further from the isoelectric point (pH=11) the structure is slightly shifted towards smaller wave-vectors (larger distance in the real space), may reflect an increase in the repulsive contribution that may be expected due to the increase of the net charge. The broadening of the peak observed at smaller pH and the consequent increase in the osmotic compressibility, may be caused by a larger degree of disorder present in the structure in this case, but could be also ascribed to a different ionic strength corresponding to the two pH. Other experiments are planned to clarify this points.

As for the case pH=6.7 at the three concentrations, we observe a large increase of the intensity for $k \rightarrow 0$. In this case the pH of the solutions is closer to the isoelectric point, and an unstructured aggregation take places, due to the dominance of the attractive part in the interaction potential. In order to have deeper information on the way that the scattered intensity passes from structured to unstructured situations, a more detailed study with smaller pH steps is undertaken.

Figure 2; Scattered intensity from a 0.10w/w lysozyme solution at pH=6.7. Five different temperature are reported.

The effect of the temperature (from 10°C to 50°C) on the scattered intensity at pH=6.7, is shown in fig.2 for the 0.10w/w concentration, the other concentrations being the same except for a scale factor. In that case the enhancement of the intensity at small wave-vector is reduced as the temperature increases. It seems therefore that an increase in the thermal energy prevents a stable aggregation among macromolecules.

From this preliminary study only some general conclusions can be drawn. The competition between the attractive and the repulsive part of the potential is driven by the pH that controls the charge distribution changes, the dipolar moments and the net charge. This competition gives rise to a minimum in the potential that leads to structured or unstructured solutions, depending on the distance of the pH from the isoelectric point.

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