A light scattering study of interactions of oppositely charged proteins in solution

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ABSTRACT

In experiments involving electrophoresis of proteins in gels, it was observed that the mobility of FITC tagged albumin (FITC albumin) was greater than that of TRITC tagged albumin (TRITC albumin). To further understand the effects of tagging proteins with fluorescent dyes, interactions of anionic proteins FITC albumin and untagged bovine serum albumin (BSA), with cationic protein poly-L-lysine was studied using dynamical light scattering. It was found that aggregates formed by the interaction of FITC albumin with poly-L-lysine were larger than those formed by the interaction between poly-L-lysine and BSA. Using zeta potential measurements it was observed that irrespective of the fluorescent tags attached to them, the zeta potential values of cationic proteins changed from negative to positive with increasing amounts of poly-L-lysine. It was also observed that addition of small amounts of poly-L-lysine to solutions containing FITC albumin decreased the zeta potential drastically. To explain this data, we are proposing a model that suggests that low concentrations of poly-L-lysine serve as scaffold-like structures on which several FITC albumin molecules anchor. We conclude that FITC appears to change the surface charge of albumin significantly and thereby influencing its behavior in solution and its interaction with cationic poly-L-lysine.

INTRODUCTION

Proteins are usually tagged with fluorescent dyes. Modification of the surface of proteins by the fluorescent tags might influence the interaction of the protein with other molecules. This might lead to effects that might be beneficial or adverse. Here we investigate the effects of tagging albumin with FITC by studying its interaction with positively charged poly-L-lysine. Albumin was chosen as the anionic protein and poly-L-lysine was chosen as the cationic protein. Albumin is a globular water soluble protein with 55% α-helical and 45% disordered structure and has an Isoelectric point of 4.7 [1 – 2]. Poly-L-lysine is a positively charged synthetic amino acid polymer which is used for promoting cell adhesion to solid substrates [3]. Poly-L-lysine forms complexes with DNA and has applications as a gene delivery tool [4]. The chemical structure and the conformation of poly-L-lysine in solution have been well characterized [5]. Also, Poly-L-lysine has been found to form large micron sized aggregates with gold nanoparticles [6]. FITC is among the most widely used fluorescent labeling reagent used due to
the fluorophore’s high quantum efficiency and conjugate stability, it has a molecular weight of 389.4, an absorption maximum at 495 nm and emission maximum at 525 nm [7-10]. We investigate the zeta potential modifications and the size changes associated with protein-protein interactions in solutions using zeta potential measurement techniques and dynamic light scattering techniques, respectively.

EXPERIMENT

Proteins

The fluorescently labeled proteins FITC (Fluorescein isothiocyanate) labeled albumin (mol wt 66 kDa) with 7 - 10 mol of FITC (mol wt 389.4) per mol of albumin (FITC albumin), Poly-L-lysine (mol wt 67.6 kDa) and BSA (Bovine serum albumin) of mol wt 66 kDa, were purchased from SIGMA. The fluorescent tags were coupled to the proteins through the ε- amino group of the lysines of the proteins.

Electrophoresis

NuSieve GTG Agarose (a low melting temperature agarose) was purchased from Cambrex Bio Science Rockland. The electrophoresis experiments were carried out in 100mM potassium phosphate solution at pH 7.0. Albumin has an isoelectric point (pI) value of ~ 4.5, and hence is negatively charged (anionic) at pH 7.0. Poly-L-lysine has a pI value of ~9.2 and a positive charge (cationic) at pH 7.0. For the electrophoresis experiments, the wells were loaded with a maximum of 20µL of the protein solution along with 20% by volume of glycerol.

Measurement of the size of aggregates in solution

In order to study the size distribution of proteins in solution, 100mM potassium phosphate solution containing proteins at concentrations of 1mg per ml were placed in 4 ml polystyrene cuvetes. To one protein solution of concentration 1 mg/ml varying volumes of another protein solution of concentration 1 mg/ml was added. The samples were gently mixed using a pipette, and the size distribution of the protein aggregates in a solution was studied using a Brookhaven Zeta plus dynamical light scattering (DLS) system equipped with a BI-9000AT digital autocorrelator at 659 nm wavelength. All studies were done at 90° scattering angle and temperature controlled at 25 °C. The number of runs for each sample was set at 10 and the run duration was set as 1 min using the software package “9KDLSW”. The correlation function was interpreted using the algorithms NNLS, CONTIN and EXPSAM.

Zeta Potential Analysis of proteins in solution

Zeta potentials were calculated using phase analysis light scattering (PALS), a variation of electrophoretic dynamic light scattering (DLS) [11]. Smoluchowski limit (i.e., κa >> 1) where “κ” is the Debye-Huckel parameter and “a” is the particle radius in Henry’s equation was applied to calculation of Zeta potential from electrophoretic mobility measurements. A dip-in electrode system with 4 ml polystyrene cuvettes was used. The electric field applied was ~ 5.1 Vcm⁻¹. The background electrolyte was chosen as 25mM potassium phosphate. All studies were done at a
controlled temperature of 25°C. The number of runs for each sample was set as 10 and the number of cycles for each run was set as 10.

DISCUSSION

Electrophoretic mobility of proteins in agarose gel

For a 2% gel run in 100mM potassium phosphate at pH 7.0, the mobility of FITC albumin and FITC poly-L-lysine were found to be $12 \times 10^{-5}$ cm$^2$V$^{-1}$s$^{-1}$ and $7 \times 10^{-5}$ cm$^2$V$^{-1}$s$^{-1}$, respectively. The mobility of FITC in 2% agarose gel was found to be $\sim 1.5$ times that of FITC albumin. This suggests that FITC is more negatively charged when compared to albumin and that tagging albumin with FITC might increase the surface charge of albumin molecule.

Size distribution of particles studied using DLS

Of particular interest in this study is the interaction of FITC albumin and BSA with poly-L-lysine when the mass concentration of poly-L-lysine is less than 5%. Using DLS it was found that as the concentration of poly-L-lysine increased the size of the FITC albumin/poly-L-lysine aggregates increased. For mixtures containing FITC albumin and 20% of poly-L-lysine, it was observed that the size of the aggregates were as large as $\sim$1 micron [12]. Figure 1 show the size distributions of particles when poly-L-lysine was added to solutions of BSA and FITC albumin. From Figure 1 it is observed that the size of the particles remains nearly unchanged for poly-L-lysine concentrations of less than 1.5% and increases to $\sim$ 100 nm as the concentration of poly-L-lysine increases to 5% in BSA rich solutions. It is observed that the size of the particles increases linearly with increasing amounts of poly-L-lysine and it reaches $\sim$ 150nm for a poly-L-lysine concentration of 5% in FITC albumin rich solutions.

![Figure 1: Size of aggregates as a function of poly-L-lysine concentrations in BSA and FITC albumin rich solutions.](image-url)
Effective diameter and particle count rate of the particles

The effective diameters of FITC albumin and BSA are ~12nm and ~12nm while that of poly-L-lysine is ~17nm. Figure 2 shows the variation in effective diameter with increasing concentrations of poly-L-lysine in BSA and FITC albumin rich solutions. From Figure 2, it is observed that the effective diameter for addition of poly-L-lysine to FITC albumin solutions increases to ~400 nm very rapidly and remains steady (400-250nm) after that. This suggests that the binding between FITC albumin and the lysine groups in poly-L-lysine molecules is tighter than the binding between BSA and the lysine groups in poly-L-lysine. Figure 3 shows the particle count rate distributions when poly-L-lysine was added to solutions of BSA and FITC albumin. From Figure 3 it is observed that the particle count rate for poly-L-lysine / FITC albumin mixtures is more than that of poly-L-lysine/BSA mixtures. This indicates that aggregation is greater in poly-L-lysine / FITC albumin mixtures than in poly-L-lysine / BSA mixtures.

Zeta potential measurements:

The zeta potential value for BSA, FITC albumin and poly-L-lysine in 25Mm potassium phosphate solutions of Ph 7.0 are -14, -15 and 15 mV respectively. Figure 4 shows the zeta potential values when varying amounts of poly-L-lysine (<5% by mass) was added to BSA and FITC albumin. It is observed that when poly-L-lysine was added to BSA, the zeta potential did not change much. When varying amounts of poly-L-lysine were added to solutions containing FITC albumin, the zeta potential value decreased to ~ -30 mV. Hence the zeta potential value for mixtures of poly-L-lysine in FITC albumin is ~2 times than that of poly-L-lysine in BSA. This change in zeta potential value confirms that the binding of FITC albumin to poly-L-Lysine is stronger than the binding of BSA to poly-L-lysine. However the change in the zeta potential value for varying amounts of FITC albumin or BSA (5%) in poly-L-lysine rich solutions is not significantly different from that of poly-L-lysine. This could be because of less aggregation.

Figure 2 and 3: Effective diameter and Particle count rates as a function of poly-L-lysine concentrations in BSA and FITC albumin rich solutions.
occurring when BSA or FITC albumin are added to poly-L-lysine-rich solutions than when poly-L-lysine is added to BSA or FITC albumin-rich solutions.

Figure 4: Zeta potential variation as a function of poly-L-lysine concentrations in BSA and FITC albumin rich solutions.

Model for formation of aggregates when oppositely charged proteins are mixed

FITC has higher electrophoretic mobility (~1.5 times more) than FITC tagged albumin in gels. This suggests that FITC tagged albumin should have greater surface charge than untagged BSA leading to the formation of bands with positively charged poly-L-lysine molecules. Most of the lysine groups in the albumin molecule are at its outer surface. Therefore most of the tagged FITC molecule should be on the outer surface of the albumin molecule. Since a high concentration of FITC (10 mol of FITC per mol of albumin) is tagged to albumin through the positively charged lysine group and since the surface area of albumin is relatively less due to its globular shape, the surface of the albumin molecule might have become strongly electronegative due to suppression of the positive charge on the surface of albumin by FITC. When poly-L-lysine is introduced into solutions rich in BSA or FITC albumin, the negatively charged globular proteins bind to some parts of the thread shaped poly-L-lysine molecules leaving the other parts of poly-L-lysine molecule to attract other negatively charged globularly shaped proteins from the solution. The steeper increase in particle count rate, constant effective diameters and steep decrease in the zeta potential value (from -15mv to -30mv) when poly-L-Lysine is introduced to FITC albumin-rich solutions (when compared with its introduction into solutions of BSA) indicates that the binding between FITC albumin and poly-L-lysine is greater than that of BSA and poly-L-lysine. Increasing concentrations of poly-L-lysine (>20%) results in larger aggregates with mostly poly-L-lysine molecules on its surface (encapsulating the albumin molecules) with a net positive charge on it. On the other hand when BSA or FITC albumin is introduced into solutions rich in poly-L-lysine, most or all parts of the FITC albumin or BSA gets attached to the poly-L-lysine molecule leaving little available region on the surface of albumin molecule to still bind to other positively charged poly-L-lysine molecules. This therefore results in lower particle count rates, less homogeneity in effective diameters and less significant change in zeta potential values.
CONCLUSIONS

Tagging proteins with FITC might change their surface charge and thereby influence their interactions with other molecules, including other proteins. FITC has higher electrophoretic mobility than FITC tagged albumin. The formation of the aggregates is a result of modification of the surface of negatively charged proteins by the FITC molecule. Light scattering experiments (effective diameter, particle count rate and particle sizes) and zeta potential measurements suggest that the binding between FITC albumin and poly-L-lysine is stronger than that between BSA and poly-L-lysine. It is also observed that the degree of aggregation is greater when poly-L-lysine is introduced into FITC albumin-rich solutions of BSA rich solution than in the reverse case of adding FITC albumin to poly-L-lysine rich solution. This is attributed to the difference in the shapes of the two proteins. A model is proposed to understand the formation of aggregates based on the modification of the surface charge of albumin by FITC and the shapes and size differences between the proteins.

ACKNOWLEDGMENTS

The particle size and zeta potential measurements were carried out in the laboratory of Dr. Martin Schoonen using systems supported by the Center for Environmental Molecular Sciences (CEMS) funded by the National Science Foundation, contract number CHE0221934.

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