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Reverse Micellar Extraction of Proteins: A Molecular Dynamic Study

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ABSTRACT

A two-dimensional mathematical model is used to elucidate the mechanism of protein transfer from an aqueous phase to a reverse micellar organic solution. The model gives the relative degree of encapsulation of a protein by the interface in response to the electrostatic interactions between them. The effect of parameters, such as charge density and charge distribution on the protein, ionic strength of the solution, and size of the protein and initial distance of the protein from the interface, on the deformation of the interface and relative mass-transfer was studied. The model qualitatively predicts the general trends of protein extraction into reverse micellar solutions.

Key Words: Reverse micellar extraction; Electrostatic interactions; Proteins; Extraction.

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INTRODUCTION

Biotechnology needs novel and efficient separation techniques in downstream processing, particularly for macromolecules, to allow continuous operation and for easy scale-up. Liquid–liquid extraction using reverse micellar organic solutions is one such technique to purify proteins.[1–5] The reverse micellar extraction consists of forward extraction of proteins from an aqueous solution to a reverse micellar organic solution. The proteins can be back extracted into a fresh aqueous phase under appropriate conditions.

The bulk aqueous phase conditions, such as pH, ionic strength, and the type of salt, govern the distribution of proteins between the reverse micellar organic phase and the aqueous solution.[6–7] The concentration and type of surfactant, presence of cosurfactant, and type of solvent decide the capacity of the reverse micellar phase.[8] The partitioning depends on the specific characteristics of the proteins, namely, the isoelectric point, size and shape of the protein, charge density and distribution on the surface of the protein, and its hydrophobicity.[9–10]

It is accepted that the driving force for the protein extraction is the electrostatic interaction between the charged polar heads of surfactant molecules and oppositely charged groups on the protein surface.[11–13] The experimental observations also suggest the necessity of increased electrostatic interactions in the case of large size proteins.[10]

Besides electrostatic interactions, hydrophobic effect also plays an important role in the uptake of proteins by the reverse micellar phase. Proteins with hydrophobic surface regions exhibit an anomalous behavior, being extracted to the reverse micellar phase even at unfavorable pH or at high salt concentrations.[6,14]

There have been relatively few studies on the modeling of mass transfer in reverse micellar systems. To date, no consistent theory is available for the characterization of protein in reversed micellar media, from the point of view of predicting the mass-transfer rate of protein-partitioning toward the organic phase. An early attempt was made by Hatton and co-workers[9] by considering protein as a single charge species. However, considering that a protein molecule is much bigger than other ionic species, it should be treated as a polyelectrolyte with multiple charges on the surface.

In this work, an attempt was made to develop a mathematical model for the extraction process of a protein molecule from an aqueous phase into the reverse micellar organic phase. As a first stage of the model development, a two-dimensional case was considered. The model provides information about the relative mass transfer of protein from aqueous phase into the organic phase as a function of system parameters, such as size of the protein, number of charges and charge distribution on the protein molecule, ionic strength of the aqueous solution, and the initial distance of the protein from the interface. Since the electrostatic interactions are primarily responsible for the protein transfer, these parameters are considered as important ones to affect the extraction process.

THE MODEL

The schematic representation of the system is shown in Fig. 1 as a two-dimensional model. The system contains an organic phase and an aqueous phase. A total of five components, water, organic solvent, salt, sodium di(2-ethylhexyl) sulphosuccinate (AOT) as surfactant molecules and a globular protein molecule are considered in a two-dimensional plane. The surfactant molecules are assumed to be completely dissociated and are located at the interface with their negatively charged headgroups. The counter ions of AOT are dispersed randomly in the aqueous solution along with NaCl in

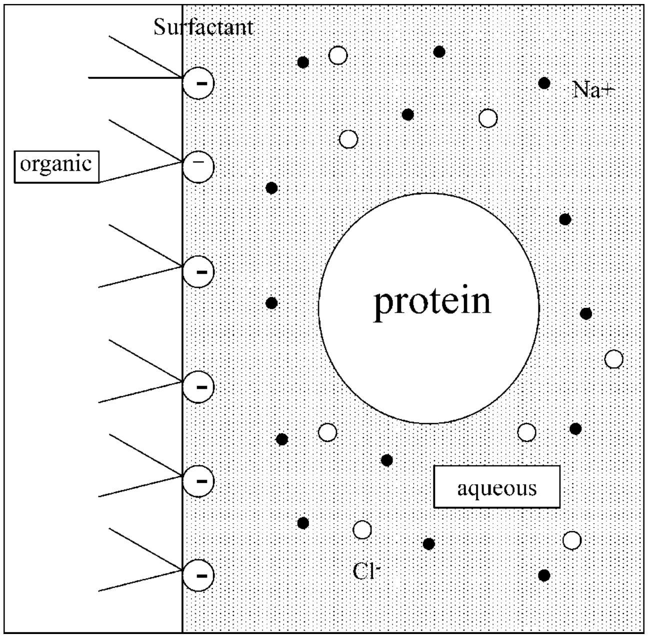


Figure 1. Schematic representation of the system.

completely dissociated form. Water and organic solvent are treated as continuous media.

Since the protein surface carries different amino acid residues of different pKa values, at a given pH, some of the residues may be positively charged and others may be negatively charged. Although the net charge on a protein molecule is considered in the experimental conditions, we considered the discrete charges as each one of them can contribute to the electrostatic interactions with other species in the environment of the protein. In the first stage, the protein molecule was considered to have uniformly spaced positive charges on its surface. Later both positive and negative charges were placed on the surface of the protein. A three-dimensional mathematical model would be required to represent the system more precisely. However, with the available resources, it was thought to simplify the problem into a two-dimensional case. In the present case, a globular protein takes a form of a circle with charges placed on its circumference. Most enzymes of industrial relevance are globular proteins and this representation is more appropriate for enzyme extraction.

The model was simplified on the basis of certain assumptions. The protein was assumed to be a rigid body. Since, usually the protein concentration is low, interaction between two protein molecules was neglected. The counter ions of surfactant molecules and the electrolyte ions were considered as point charges for the force calculations between them.

Molecular dynamics was considered as an approach for characterizing the mass-transfer process, which complements and helps to explain the existing data on the mass-transfer aspects.[4] The simulation involved estimating the velocities of molecules and ions based on their positions and interspecies forces at time t. The net force, F_i, on each species provides its acceleration, a_i, which in turn, gives the changes in its velocity and position. The procedure was repeated at the new positions of all species in the system after a time step Δt. The calculation allowed tracing the trajectory of a molecule over a period of time with respect to the interface.

For the integration of Newton's laws of motion involved in the molecular dynamics simulation, a discrete time step of 0.1 psec was used for all the cases, except for the case when ionic strength of the solution was 0.02 mol/dm^3, where a time step of 0.2 psec was found suitable.

The motion was considered in Stokes region for the estimation of the drag force by the solvent molecules. The drag coefficients were considered on the basis of the size difference between the diffusing species and water molecules.[15] For the surfactant molecules, in addition to the electrostatic and drag forces of solvent, surface tension force was also added to resist the interfacial deformation, which reduces their mobility into aqueous phase.

The extent of deformation of the interface was considered in terms of the positions occupied by the surfactant molecule's headgroup. No transfer of counter ions was permitted into the organic phase.

RESULTS AND DISCUSSION

Molecular dynamics simulation was carried out for a 40 nsec period to understand the mechanism of transfer of the protein from aqueous phase to organic phase. The radius of the protein was taken to be 25 Å. The protein was at a distance of 5 Å from the interface initially. Three positive charges and one negative charge were placed on the circumference of the protein randomly. The ionic strength of the aqueous phase in terms of NaCl concentration was 0.01 mol/dm^3.

Figure 2 shows the position of the protein molecule and the extent of deformation of the interface as the molecular dynamics simulation progressed with respect to time. The protein is represented by a circle and the positions of the surfactant headgroups are denoted by points on the deformed interface. The interface itself is considered on lines joining the adjacent surfactant headgroups. As the simulation progressed, it was observed that the protein was moving toward the interface due to the electrostatic interactions between the protein charges and the charges on the headgroups of the surfactant molecules at the interface. However, the migration rate of the surfactant molecules, which attracted toward the protein and, therefore, the deformation of the interface, were much greater than the migration rate of the protein toward the interface, as indicated by the displacement of the surfactant headgroups toward the right of the original position. The interface started deforming much more as the protein approached closer to it. The deformation of the interface closer to the protein was the highest. As the protein moved closer to the interface, the section of the interface closest to the protein was pushed backward into the organic phase, while the interfacial surface farther away on either side was attracted toward the protein, enveloping part of the protein with a certain amount of the aqueous phase. The net result was that the interface started enveloping the protein. In 25 nsec, around 90% of the protein was encapsulated. After that, the relative speed of the protein and the rate of its encapsulation reduced considerably, indicating that the complete encapsulation forming a protein loaded reverse micelle is the controlling step in the transfer of a protein from the aqueous phase to the organic phase. The same trend was observed throughout the other simulation runs with different parameters.

To observe the extent of deformation and the relative degree of encapsulation with respect to the charge density on the protein, the charge

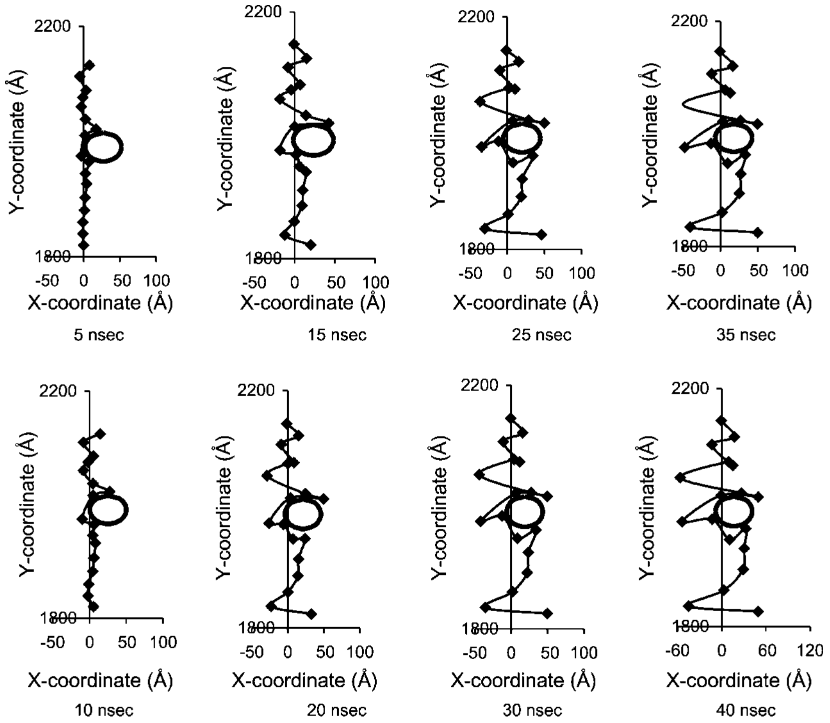
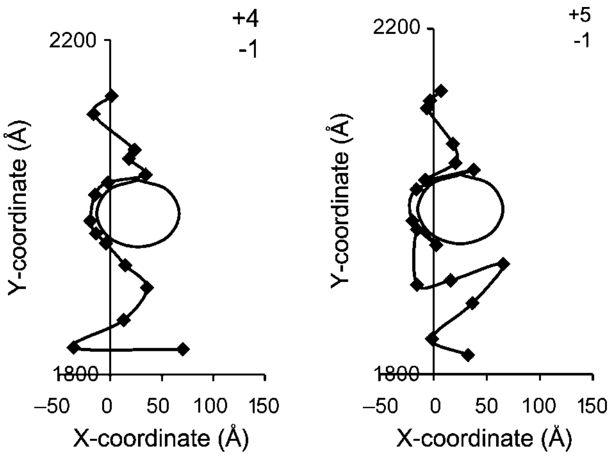
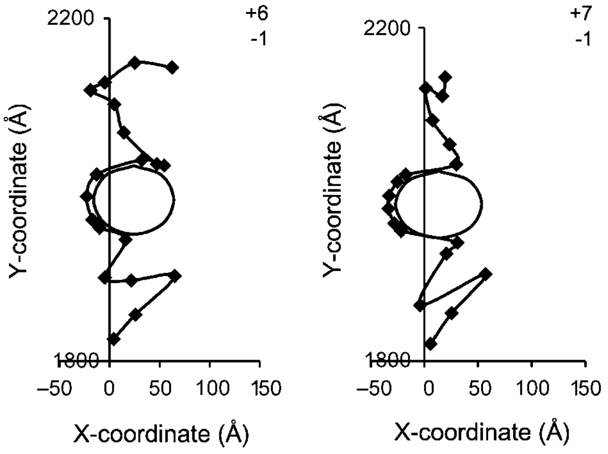


Figure 2. Extent of deformation of the interface and the position of the protein as simulation progresses (radius of the protein = 25 Å, ionic strength = 0.01 mol/dm^3).

density was increased from 1.925×10^{-11} C/m (+3 net charge on the protein) to 3.848×10^{-11} C/m (+6 net charge on the protein). Figure 3 shows the simulation results for a protein of 40 Å radius. With the increased charge density, the attractive force between the protein and the surfactant molecules increased, leading to an enhanced relative mass-transfer rate (in terms of the average velocity of the protein) and a higher degree of encapsulation with time. Average velocity of the protein was defined as the distance traveled by the protein in x-direction divided by the time required. The protein traveled the largest distance toward the organic phase when the net charge on it was +6, with around 80% encapsulation. The degree of encapsulation of the protein was defined as the percentage area of the protein covered by surfactant molecules on the interface. Experimental results have shown higher mass-transfer rates with anionic surfactants for proteins at pH below their isoelectric point with increasing net charges.[6,16]



Net Charge = +3 **Net Charge = +4**



Net Charge = +5 **Net Charge = +6**

Figure 3. Molecular dynamics simulation after 40 nsec for different charge densities on the protein (radius of the protein = 40 Å, ionic strength = 0.01 mol/dm^3).

Keeping the charge density on the protein constant (2.052×10^{-11} C/m), when the size of the protein was increased from 25 Å to 60 Å, the relative mass transfer decreased with the increasing protein size (Fig. 4). For a protein with 25 Å radius, the relative encapsulation of the protein was higher than that with

40 Å radius. At this stage, the interface covered around 95% area of the protein. For the protein with 60 Å radius, however, only about 45% encapsulation of the protein was observed during the same time. Further encapsulation of a large size protein, therefore, should take much longer time,

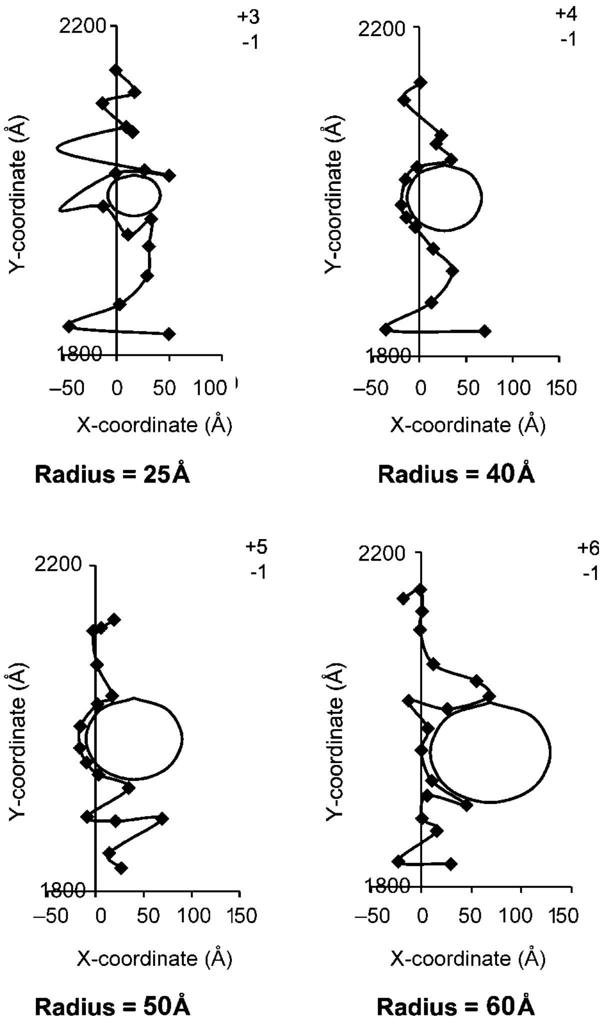


Figure 4. Molecular dynamics simulation after 40 nsec for different sizes of the protein (ionic strength = 0.01 mol/dm^3).

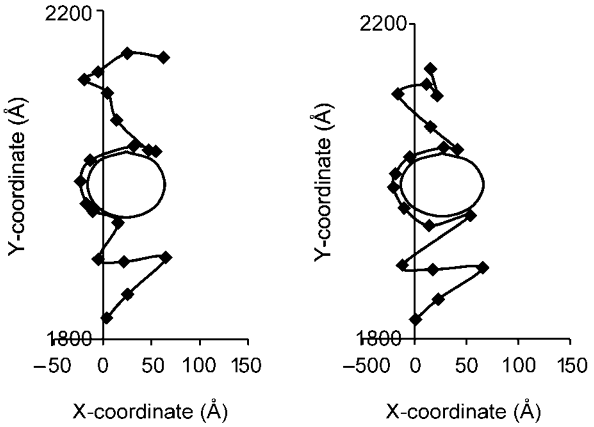
as was observed even for a small size protein. Similar results also have been observed experimentally.

Even though the net charge on the protein was kept constant at +5, the distribution of positive and negative charges on the protein had an effect on the net transfer of the protein. The individual number of positive and negative charges was varied on a protein of 40 Å radius. The extent of deformation of the interface and the position of the protein for four different charge distributions are shown in Fig. 5. The encapsulation was around 60% for the case with six positive and one negative charge on the protein. In all other cases, the encapsulation was around 70%. The displacement of the interface shown by the position of the surfactant headgroups is much larger than that of the protein molecule moving toward the organic phase. It indicates that the surface mobility is more important than the net velocity with which the protein is moving.

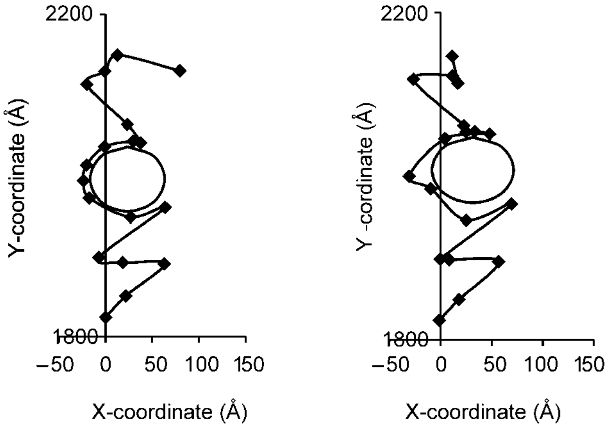
Since the major forces of interactions are electrostatic in nature, the added salt screens the attractive or repulsive interactions between the charged surfaces and thereby reduces the distance over which the attraction or repulsion is felt. To investigate the effect of ionic strength on the deformation of the interface and the relative degree of encapsulation of a protein, the ionic strength of the aqueous solution was varied from $0.005\,\text{mol/dm}^3$ to $0.02\,\text{mol/dm}^3$. When the ionic strength of the aqueous solution was $0.02\,\text{mol/dm}^3$, the electrostatic attraction between the protein and the oppositely charged surfactant headgroups reduced so significantly that net movement of the protein was away from the interface, toward the bulk aqueous phase, and a very slight deformation of the interface was observed. It was expected that the protein movement would be slower toward the interface when the ionic strength was increased from $0.005\,\text{mol/dm}^3$ to $0.01\,\text{mol/dm}^3$ because of the screening effect of the counter ions associated with the interface and the protein surface. The surface area of the protein covered by the interface was around 70% when the ionic strength was $0.005\,\text{mol/dm}^3$, as compared to 60% when it was $0.01\,\text{mol/dm}^3$ during the same time (Fig. 6).

Molecular dynamics simulation was carried out for three different cases in which the initial point of placement for the protein with 40 Å radius was 5 Å, 8 Å, and 11 Å from the interface. The positive and negative charges on the protein were 6 and 1, respectively. The simulation results for the three cases are shown in Fig. 7.

As the initial distance between the protein and the interface increased, the attractive force between the protein and the oppositely charged surfactant molecules decreased, resulting in reduced rate of migration of the protein. In all the three cases, however, the percentage encapsulation of the protein was



Charges = +6 & -1 **Charges = +7 & -2**

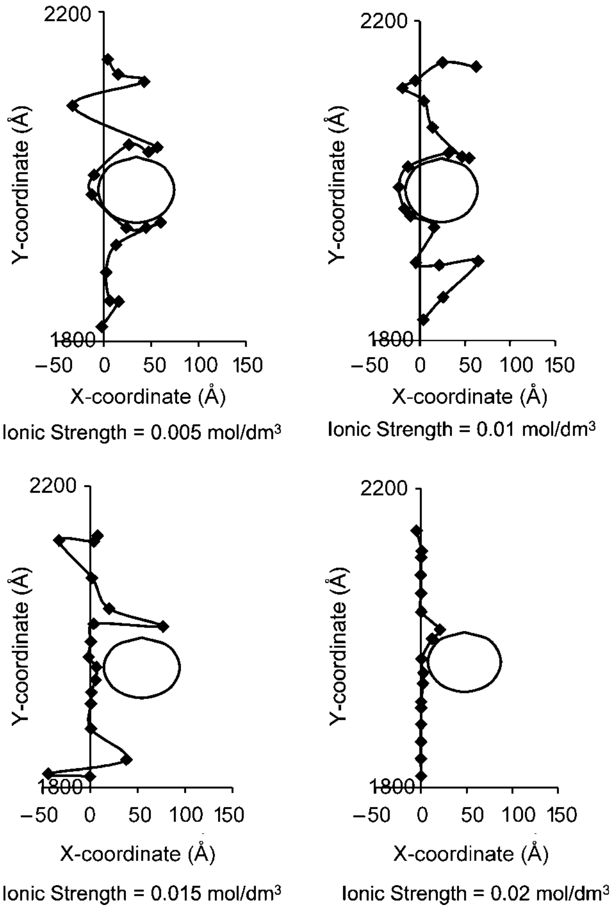


Charges = +8 & -3 **Charges = +9 & -4**

Figure 5. Molecular dynamics simulation after 40 nsec for different charge distributions on the protein (radius of the protein = 40 Å, net charge on the protein = +5, and ionic strength = 0.01 mol/dm^3).

around 60%, suggesting a higher surface mobility as compared to the migration rate of the protein.

Although the two-dimensional model could predict the trends observed experimentally,[17–19] it was not able to estimate the true mass-transfer coefficient. A three-dimensional model, therefore, needs to be developed for



Ionic Strength = 0.005 mol/dm^3

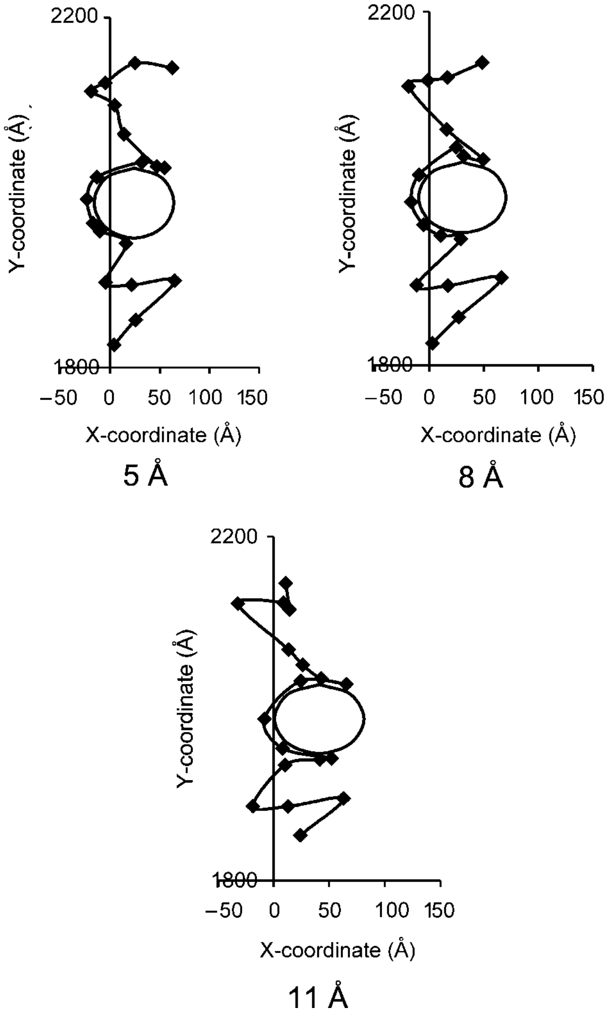
Ionic Strength = 0.01 mol/dm^3

Ionic Strength = 0.015 mol/dm^3

Ionic Strength = 0.02 mol/dm^3

Figure 6. Molecular dynamics simulation after 40 nsec for different ionic strengths of the aqueous solution (radius of the protein = 40 Å, net charge on the protein = +5).

precise interpretation of the system. Hydrophobic interactions between the hydrophobic patches on the protein and the hydrophobic tails of the surfactant molecules also need to be included in the model. The model considers only those surfactant molecules that are at the interface, and neglects those in the bulk organic phase. Generalization of the model to consider any type of electrolyte will be more useful.



5 Å

8 Å

11 Å

Figure 7. Molecular dynamics simulation after 40 nsec for different initial distances of the protein from the interface (radius of the protein = 40 Å, net charge on the protein = +5, ionic strength = 0.01 mol/dm^3).

CONCLUSION

Mechanism of transfer of protein during forward extraction is elucidated by considering the deformation of surfactant-laden liquid–liquid interface around a protein molecule. Charge interactions between the protein and the surfactant molecules produce a significant deformation of the interface leading to protein envelopment by the interface.

The relative mass transfer for the reverse micellar extraction increases with the increased charge density on the protein and greater electrostatic interactions are required to extract a larger protein. Along with the charge density on the protein, the charge distribution on the protein and ionic strength of the aqueous phase play an important role in the extraction process. Increased ionic strength of the aqueous solution screens the electrostatic interactions leading to a reduced mass-transfer rate.

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