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Counterion effects in protein nanoparticle electrostatic binding: A theoretical study

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ABSTRACT

Effects of counterions on the folding conformation of proteins, bound electrostatically on the surface of charge-ligand functionalized nanoparticles, have been investigated based on the protein folding energy calculation. The folding energy of a protein has been taken as a sum of the short range interaction energies, like, the van der Waals attraction and the hydrogen bond energies, and the long range coulomb interaction energy. On electrostatic binding, counterions associated with surface ligands of nanoparticles diffuse into bound proteins through the medium of dispersion. As a result, bound proteins partially unfold, as observed in circular dichroism experiments, which has been realized using the "charge-dipole" and the "charge-induced dipole" interactions of counterions with polar and non-polar residues, respectively. The effect of counterions solvation in the dispersing medium, e.g., water, which causes water molecules to polarize around the counterions, has also been considered. The folding energy of bound proteins has been seen to decrease proportionally with the increasing number of diffusion of counterions and their polarizability.

1. Introduction

Study of the protein–nanoparticle interaction is important on the ground of applications of functional nanoparticles in medical therapies; e.g., drug delivery [1,2], magnetic resonance imaging (MRI) [3,4], hyperthermia treatment for tumor cells [5–7], and so on. Upon intra venous administration of nanoparticles, proteins get adsorbed at the nano-bio interface mediated by forces; like, solvation forces, hydrophobic attraction and electrostatic interactions; and thus form a dynamic 'corona' on the nanoparticle surface. These bound proteins may become biologically toxic. Therefore, the toxicity of nanoparticles is one of the major concerns for biomedical applications [8–10]. We have earlier reported [11–13] that charge-ligand functionalized nanoparticles electrostatically bind with the oppositely charged proteins. As a result, counterions associated with the ligands on nanoparticle surface become "sterically" free to diffuse into bound proteins and unfold their secondary conformations. This sort of interaction model has been given a name by us as the "reverse-charge-parity counterions" or RCPC model [13].

Proteins are polypeptides of amino acids having well defined folding conformations and carrying a net surface charge depending on the pH of the dispersing medium. They fold spontaneously into complicated three-dimensional structures that are essential for specific biological activities. Understanding the protein folding mechanisms helps to design and modify the novel proteins, to understand human degenerative diseases caused by protein misfolding and/or aggregation [14,15]. A number of different interactions define the protein folding conformations. These include hydrogen bonds, electrostatic interactions, van der Waals interactions and hydrophobic interactions [16,17]. As protein folds in an aqueous environment, contribution of a specific interaction depends on the difference between the interactions within the protein (interior) and the interaction of the protein with the adjacent water molecules (exterior). For example, both the hydrogen bonds and the van der Waals interactions occur within the folded protein, as well as between the solvent molecules and the protein residues. On the other hand, both the electrostatic and the hydrophobic interactions have significant contributions within the protein and play specific roles in the folding of proteins [18]. Therefore, the stability of a protein solution depends on several factors, like, the macromolecular net charge, the solvent pH, the chemical nature of the dissolved ions [19], and so on.

The ion binding with protein residues changes its folding conformation. For example, binding of Ca^{2+} ion to the transglutaminase causes an increase of its radius of gyration, indicating the unfolding of the protein [20,21]. Binding sites of the metal ions in a protein are varied in their coordination numbers and geometries, their metal preferences, and their ligands (which include backbone carbonyl oxygen; side chain groups and water molecules) [22,23]. Yamashita et al. [24] have reported that the metal ions generally bind in the

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regions of high hydrophobicity contrast in proteins which is due to the fact that the electron distributions in the metal ions are highly symmetric, attracting the electron-pair donors (Lewis bases) around the ion in a shell. In proteins these electron-pair donors are oxygen, nitrogen, and sulfur atoms. Interactions of metal ions with the carboxylic and the carboxamide groups in protein structures have also been reported [25,26]. Protein surfaces are far from uniform, consisting rather of an intricate network of polar and non-polar groups to which salt ions have widely different affinities. For example, large anions are attracted to the hydrophobic interfaces (Hoffmeister effect) via the surface modified solvation and the polarization [27,28]. Direct ion-pairing [29] between salt particles and charged surface groups also give rise to ion specific phenomena [30].

Protein design presents a demanding task for a potential energy function. The energy produced by design potentials is intended to correlate with the free energy of protein folding. The force field also must be compatible with the computational requirements of protein design. For example, energy terms must be pair wise decomposable. In this work, the folding energy of native proteins has been calculated based on interactions involving the van der Waals force between polar and non-polar residues, the hydrogen bonding between polar residues, and the electrostatic attraction between charged residues [16]. Aim of this work was to calculate the change in the folding energy of proteins, on electrostatic binding with the charge-ligand functionalized nanoparticles and subsequent diffusion of counterions, leading to their partial unfolding [11–13]. This is the first theoretical work using the RCPC interaction model [13], between charged proteins and oppositely charge-ligand functionalized nanoparticles, which has explained possible mechanisms of unfolding of bound proteins due to the diffusion of counterions.

2. Method and calculations

2.1. Protein folding energy

2.1.1. van der Waals energy

The most significant packing force of folding of a protein involves the short range van der Waals potential between both polar and non-polar residues. This potential provides a physical basis for the side chain packing specificity, thereby supporting the native-like folded state with well-organized cores, and is given by the Lennard–Jones 12-6 expression [16],

$$E_{vdW} = \left(\frac{D_0}{4\pi\varepsilon\varepsilon_o}\right)\left[\left(\frac{r_0}{r}\right)^{12} - 2\left(\frac{r_0}{r}\right)^{6}\right] \quad (1)$$

where r is the distance between the center of a pair of interacting atoms (within polar and/or non-polar residues) and the center of the protein core, and has been computed using the protein specific atomic coordinates obtained from different protein data banks. r_0 is the equilibrium radii of the core and D_0 is the depth of the potential well which was taken 8 kJ in the present calculations. ε and ε_o are the dielectric constants of water (=78.4) and vacuum (=1), respectively. In the present calculation, it was assumed that the van der Waals interaction between non-polar residues would be equivalent to the hydrophobic interaction, as the later one also varies inversely with 6th power of r [11].

2.1.2. Hydrogen bond energy

The energy, E_{HB}, of hydrogen bonding (H-bond) between polar residues of a protein is given by [16]

$$E_{HB} = \left(\frac{D_0}{4\pi\varepsilon\varepsilon_o}\right)\left[5\left(\frac{r_0}{r}\right)^{12} - 6\left(\frac{r_0}{r}\right)^{10}\right]F(\theta) \quad (2)$$

The expression for $F(\theta)$ depends on the type of hybridization used in donor and acceptor atoms. In this calculation, sp^3–sp^3 hybridization was taken into consideration (for simplicity) which gives, $F(\theta) = \cos^4\theta$ [16]. As the average over angular distributions, $<\cos^2\theta> = 1/3$, we get, $F(\theta) = 1/9$. Note that the contribution of this term appears in designing the helical surfaces [31].

2.1.3. Electrostatic energy

The electrostatic interaction arises due to the charge residues in protein and is not strong enough to compensate for the energy of desolvation [32]. It only maintains the specific folding of protein, and its functional interactions [33,34]. A simple form of the electrostatic energy, E_{ES}, is given by the distance-attenuated Coulomb interaction term [16],

$$E_{ES} = 322.0637\sum_{ij}\left(\frac{Q_iQ_j}{4\pi\varepsilon\varepsilon_o r_{ij}}\right) \quad (3)$$

where Q's are charges of ith and jth atoms separated by a distance r_{ij} which can be calculated using the coordinates of respective atoms. Contribution of this term to the total protein folding energy is significant only when the charged atoms are in close proximity. It is to be noted here that close to the charge surface of protein the water molecules are polarized, and a favorable interaction between water dipoles and protein charge maintains the stability of protein dispersion.

2.2. Protein–nanoparticle RCPC interaction and counterions effects

As mentioned earlier, contribution of the electrostatic interaction to protein folding is not significant. On electrostatic binding with the charge-ligand functionalized nanoparticle, charge residues of proteins can no longer maintain their specific folding conformation. In addition, counterions associated with the surface ligands of nanoparticles get condensed around protein surface, further assisting the diffusion of counterions into the protein interior. As a result, folding energy due to the van der Waals interaction (Eq. (1)) and the hydrogen bonding (Eq. (2)) would reduce. Counterions in the interior of protein would, on the other hand, develop attractive interactions with both polar and non-polar residues. As a result of these attractive interactions of protein residues with external agents, like counterions, would reduce the intra-protein short range interactions. In addition, the counterion solvation in water would cause the adjacent water molecules to take polarized structure changing the dielectric constant and entropy of the dispersing medium. Therefore, the solvation of counterions may cause several effects, like, the reduction of hydrophobicity, the breaking of hydrogen bonds, etc. Details of these effects have been discussed below.

If a counterion of charge q lies at a distance r from the center of a polar residue of dipole moment μ and dipolar length l, the corresponding attractive coulomb energy, E_{dip}, can be given by [35]

$$E_{dip} = -\frac{nq\mu\cos\theta}{4\pi\varepsilon\varepsilon_o r^2} = -\frac{n(ze)\mu}{4\pi\varepsilon\varepsilon_o r^2} \quad (4)$$

where n represents the number of counterions interacting with the polar residues, z is the valency of counterions, e is the elementary charge. θ is the angle between the dipole and the line joining the counterion to the center of the dipole. For attractive interaction, $\theta = 0^\circ$. Again, the energy, E_{ind}, of attraction between the counterions and the non-polar residues of bound proteins can be given by [35]

$$E_{ind} = -\frac{n\alpha(ze)^2}{2(4\pi\varepsilon\varepsilon_o)^2 r^4} \quad (5)$$