Obtaining Protein Solvent Accessible Surface Area When Structural Data is Unavailable Using Osmotic Pressure

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Here, we provide an algorithm that predicts solvent accessible surface area (SASA) using concentrated solution osmotic pressure data. Sheep hemoglobin monomer and β-lactoglobulin are used for verification. Additionally, SASA for structurally unknown calf lens α-crystallin aggregate is predicted. Using osmotic pressure data, the predicted SASA value for sheep hemoglobin, 22,398 ± 1,244 Å^2, was in excellent agreement with computational model predictions (24,304 Å^2-26,100 Å^2). Similarly, predicted SASA values for bovine β-lactoglobulin in pH solutions of pH 5.1, 6.0, and 8.0, were 5,765 ± 1,031 Å^2, 6,656 ± 1,082 Å^2, and 9,141 ± 1,060 Å^2, respectively, were in good agreement with the computationally determined SASA value (7,500 Å^2–8,628 Å^2). Predicted SASA for the aggregate of calf lens α-crystallin (800 kDa) was found to be 417,691 ± 16,790 Å^2. These results illustrate that this novel method can provide an important experimental alternative in estimating SASA for proteins and, possibly, their complexes in solution.
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Introduction

Computational estimations of the solvent accessible surface area (SASA), first introduced by Lee and Richards,[1] has tremendous applicability in terms of understanding free energies of solvent–protein interaction, protein–protein interactions, protein folding, and many other thermodynamic properties of the protein solution.[2] Although a number of methods exist to estimate this important parameter, nearly all of them critically rely on the availability of the crystal structure of the protein of interest. Here, we introduce the use of a highly robust free-solvent model that uses osmotic pressure data from proteins at near-saturation concentration to extract SASA with accuracy equivalent to computational methods that use the protein crystal structure.

A number of models have been proposed that attempt to understand the nonideal effects of crowded protein osmotic pressure; however, most of them focus primarily on protein–protein interactions as the predominant contributor to the anomalous behavior. Nearly all of these models use a variant of the virial expansion paradigm that is based on McMillan–Mayer theory.[3] However, these models are severely constrained to dilute solutions, and more importantly, lack physically realistic parameters.

Recently, a free-solvent model was developed that accurately describes the osmotic pressure of several globular proteins and binary solutions of proteins to near-saturation.[4–8] Unique to this modeling approach is that the associated model parameters used for predicting the osmotic pressure are physically significant and can often be verified by independent methods.

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The foundation for the free-solvent model was originally developed by van Laar[9] and further by Lewis and Randall.[10] van Laar[9] proposed that the nonideal behavior of the osmotic pressure for solutes in aqueous solutions was coupled to the solvent–solute interactions. Additionally, he also argued that the appropriate concentration variable to describe osmotic pressure is the mole fraction. More recently, Yousef et al.[4–8] revised this model to incorporate ion binding and found that describing the solute in the free-solvent environment as a hydrated species containing a single monolayer of water and the influenced solvent provides excellent correlations between the observed osmotic pressure data, as well as describe the underlying mechanisms for the observed behavior of concentrated protein environments. This has been shown for both single and binary protein solutions, without any fitted parameters.

The purpose of this study is to relate the measured osmotic pressure of aqueous protein solutions to their SASA via the free-solvent model. There is limited protein solution data in the literature that reports osmotic pressure values for protein concentrations to near saturation and, simultaneously, have the molecular structure available in the Protein Data Bank (PDB) for the protein investigated. However, near-saturation osmotic pressure data and the molecular structure are available for both sheep hemoglobin (69 kDa)[11,12] and β-lactoglobulin (18.4 kDa),[13–16] which are used in this study. Additionally, although its structure has not been registered in the PDB, we will also predict the SASA of calf lens α-crystallin from reported osmotic pressure data.[17]

For all three proteins, the physically realistic parameters of the free-solvent model are regressed upon using the respective osmotic pressure data. It was previously shown that the hydration parameter correlates to a monolayer of water and, thus, corresponds directly to the respective SASA.[8] Therefore, using the regressed hydration parameter from the free-solvent model, the SASA for these proteins are determined. Given that the x-ray diffraction structure is available for sheep methemoglobin (PDB: 2QU0[12]) and bovine β-lactoglobulin (PDB: 2Q2M,[14] 3NPO,[15] and 3BLG[16]), the free-solvent based SASA for both proteins is compared with the respective values estimated from computational modeling.

Free-solvent model

The development of the free-solvent model is described elsewhere.[4] In summary, the osmotic pressure, π, for a single protein in aqueous solution with a single monovalent salt is described as

$$\pi \approx \frac{RT}{\overline{V}_1} \ln\left(\frac{\left(N_1^{\mathrm{II}} + (1 - v_{12} - v_{32})N_2^{\mathrm{II}} + N_3^{\mathrm{II}}\right)N_1^{\mathrm{I}}}{\left(N_1^{\mathrm{II}} - v_{12}N_2^{\mathrm{II}}\right)N^{\mathrm{I}}}\right), \quad (1)$$

where N_i^k is the number of moles of species i in compartment k, and v_{ij} is the net number of moles of solvent component i interacting with protein j. Subscripts 1, 2, and 3 refer to the solvent, protein, and salt, respectively. The compartment containing the protein solution is denoted as superscript II, while the nonprotein compartment is denoted as superscript I.

Solvent accessible surface area and monolayer of water

The amount of water that interacts with proteins has been extensively studied; however, exact values of hydration are difficult to determine due to the various methods used.[18] Nevertheless, the consensus, from ^{17}O NMR studies and mathematical modeling, is that globular proteins contain about 1 g H_2O/g Protein.[18] Yousef et al.,[8] using the free-solvent model, further refined this observation and showed that the hydration of all globular proteins studied in moderate monovalent salt concentrations was a single monolayer or 15.2 $\pm$ 0.5 molecules of water per nm^2 of SASA. This observation is relative to the observed osmotic pressure nonideal behavior and, thus, resulted from studies conducted at high protein concentrations, near-saturation conditions for accuracy.

Robustness of SASA estimates relative to solution properties

It has also been shown that the estimated hydration parameter (and thus the resulting SASA) is relatively insensitive to changes in ionic strength and pH, provided the proteins are in moderate ionic strength solutions. Yousef et al.[6] showed that, for concentrated ovalbumin (OVA) in aqueous solutions of pH 7.0 and ionic strengths of 0.15 M NaCl and 0.5 M NaCl, the hydration values were 0.86 g H_2O/g OVA and 0.89 g H_2O/g OVA, respectively. Similarly, Yousef et al.[5] showed that for concentrated bovine serum albumin (BSA) solutions in 0.15 M NaCl with pH values varied from 4.5, 5.4, and 7.4, the hydration values were 1.113 g H_2O/g BSA (pH 4.5), 1.137 g H_2O/g BSA (pH 5.4), and 1.177 g H_2O/g BSA (pH 7.4).

Experimental Methods

Here, the osmotic pressure data for aqueous solutions of sheep hemoglobin (molecular weight, 69 kDa), reported in the literature,[11] is regressed using the free-solvent model. The osmotic pressure experiments were conducted for protein concentrations (g/L solution), up to near-saturation, in 0.1 M KCl solutions at pH 7.43 and 0°C. The values of hydration and ion binding were then regressed to best fit Eq. 1 using nonlinear regression (TableCurve 2D (Systat Software, San Jose, CA) and Datafit (Oakdale Engineering, Oakdale, PA)). The resulting values for hydration and ion binding were compared with literature values, when available. The SASA, predicted using the osmotic pressure data, for sheep hemoglobin was compared to the calculated SASA determined from the molecular structure of sheep methemoglobin (PDB code: 2QU0[12]) using Swiss-Pdb Viewer,[19] MOLMOL,[20] UCSF Chimera,[21] and GETAREA.[22]

Literature values for the osmotic pressure data of aqueous solutions of bovine β-lactoglobulin (molecular weight, 18.4 kDa) for 0.1 M NaCl at 20°C and three pH (5.1, 6.0, and 8.0)[13] were also modeled using the free-solvent model. Similar to above, the regressed hydration and ion binding, and SASA were determined. The resulting SASA was compared with the SASA values calculated from the molecular structure of bovine β-lactoglobulin (PDB: 2Q2M,[14] 3NPO,[15] and 3BLG[16]) using Swiss-Pdb Viewer,[19] MOLMOL,[20] UCSF Chimera,[21] and GETAREA.[22]

Finally, osmotic pressure data for aqueous solutions of calf lens α-crystallin (molecular weight, 800 kDa) for 0.15 M KCl at pH 6.8 and room temperature[17] was modeled using the free-solvent model. Again, the values of hydration and ion binding were regressed using Eq. 1 and the SASA

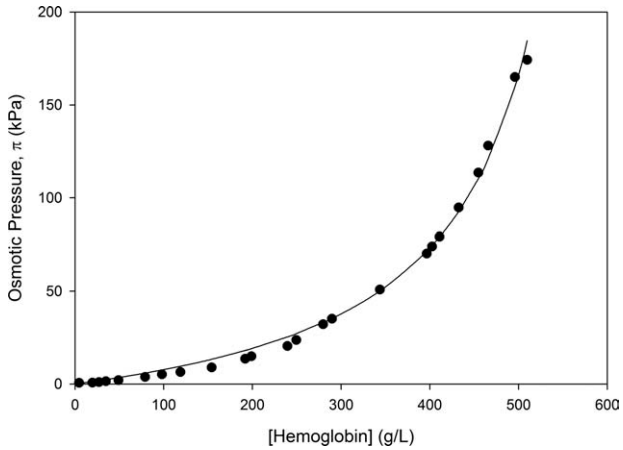

Figure 1. Osmotic pressure vs. hemoglobin concentration at 0°C, 0.1 M KCl, and pH 7.43.

The circles represent previously published data.[11] The solid curve is the free-solvent model with regressed hydration and ion binding values. The regressed hydration (v_{12}) and ion binding (v_{32}) parameters were determined to be 0.869 ± 0.0118 g H_2O/g Hb and 5.20 ± 0.135 mol KCl/mol Hb, respectively.

was determined. Because no molecular structure has been deposited in the PDB, the predicted SASA for α-crystallin was not compared with computational SASA.

Results

Predicting hydration and ion binding

Figures 1–3 show the free-solvent model applied to the osmotic pressure data obtained from the literature for sheep hemoglobin, β-lactoglobulin, and calf lens α-crystallin solution for their respective solution properties.[11,13,17] As can be seen, the free-solvent model, using the regressed parameters (solid curve), provides an excellent fit to all of the experimental data over the entire concentration range for each case. The regressed hydration, v_{12}, and ion binding, v_{32}, values for each are shown in Tables 1 and 2.

Covariance of the Regressed Parameters. The covariance between the regressed parameters, hydration and ion binding, were also determined to further validate the model form and the associated regressed parameters. A low covariance indicates parameter independence and strengthens credibility of this modeling approach. The covariance values resulting from these studies were 4.2×10^{-8} and 1.6×10^{-5} for the sheep hemoglobin and the calf α-crystallin results, respectively. The covariance values resulting from the β-lactoglobulin results were 3.9×10^{-8}, 1.1×10^{-5}, and 2.1×10^{-7}, for pH 5.1, 6.0, and 8.0, respectively. The fact that the hydration and ion binding values are physically relevant and strongly independent in the regression analysis provides a strong indication of the validity of the free-solvent model in representing the osmotic pressure behavior for concentrated protein solutions.

Predicting the solvent accessible surface area (SASA)

The free-solvent model regressed hydration values were used to predict the SASA for sheep hemoglobin, bovine β-lactoglobulin, and calf lens α-crystallin. Table 1 reports these predicted values.

Discussion

Comparison of regressed hydration and ion binding parameters to the literature

Tables 1 and 2 compare experimental values of hydration and ion binding with the regressed hydration and ion binding values used in the free-solvent model for all three proteins investigated.

Sheep Hemoglobin. The free-solvent model regressed hydration value of sheep hemoglobin (0.869 ± 0.0118 g H_2O/g Hb) is consistent with the hydration of bovine and human hemoglobin (0.51 g H_2O/g Hb) that was determined using static and dynamic light scattering.[23,24] The free-solvent model regressed ion binding value of sheep hemoglobin (5.20 ± 0.135 mol KCl/mol Hb) is in excellent agreement with chloride ion binding values (6 mol Cl^-/mol Hb bound at pH 7.4) determined for bear, horse, and bovine hemoglobin using molecular dynamic simulations.[25] To determine ion binding, two experimental methods have been extensively employed, the distribution method and the EMF method.[26–28] A major drawback of using these methods is the large error (up to 40%). Using the regressed values, the free-solvent model, given sufficiently concentrated osmotic pressure data, predicts the ion binding for sheep hemoglobin to within 3% error.

Bovine β-Lactoglobulin. The experimental value for hydration of β-lactoglobulin from ^{17}O NMR measurements, by Mattea et al.,[29] was determined for bovine β-lactoglobulin to be approximately 0.72 g H_2O/g β-lactoglobulin. This is excellent agreement with the regressed values of hydration from this study. No ion binding studies, experimental or calculated, were found for β-lactoglobulin for comparison.

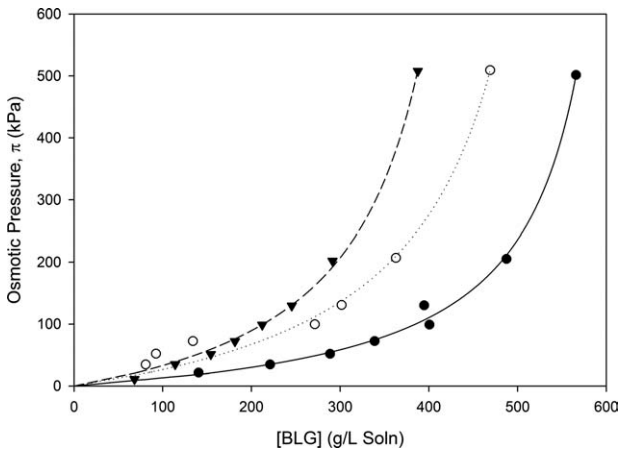

Figure 2. Osmotic pressure vs. β-lactoglobulin concentration at 20°C, 0.1 M NaCl, for three pH (5.1, 6.0, and 8.0).

The closed circles, open circles, and triangles represent previously published data for pH 5.1, 6.0, and 8.0, respectively.[13] The solid curve, dotted curve, and dashed curve are the free-solvent model with regressed hydration and ion binding values for pH 5.1, 6.0, and 8.0, respectively. The regressed hydration (v_{12}) parameter was determined to be 0.809 ± 0.0138 g H_2O/g β-lactoglobulin, 0.942 ± 0.0574 g H_2O/g β-lactoglobulin, and 1.313 ± 0.0206 g H_2O/g β-lactoglobulin for pH 5.1, 6.0, and 8.0, respectively. The regressed ion binding (v_{32}) parameter was determined to be 1.76 ± 0.068 mol NaCl/mol β-lactoglobulin, 1.17 ± 0.285 mol NaCl/mol β-lactoglobulin, and 1.57 ± 0.102 mol NaCl/mol β-lactoglobulin for pH 5.1, 6.0, and 8.0, respectively.

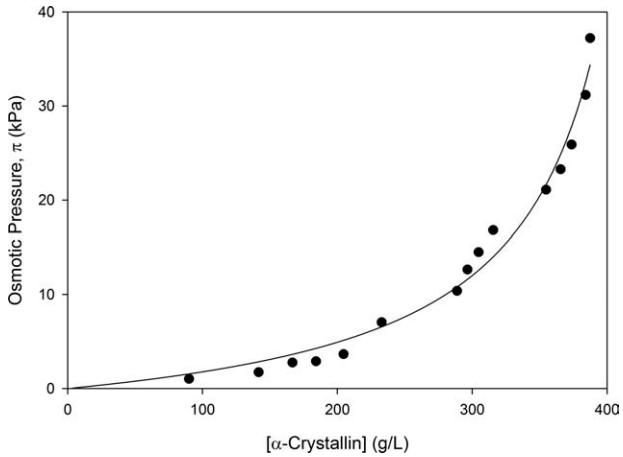

Figure 3. Osmotic pressure vs. α-crystallin concentration at room temperature, 0.15 M KCl, and pH 6.8.

The circles represent previously published data.[17] The solid curve is the free-solvent model with regressed hydration and ion binding values. The regressed hydration (ν_{12}) and ion binding (ν_{32}) parameters were determined to be 1.433 ± 0.0409 g H_2O/g α-crystallin and 169 ± 5.27 mol KCl/mol α-crystallin, respectively.

Calf Lens α-Crystallin. Calf lens α-crystallin is found as a dimer of two different subunits, αA-crystallin (αA) and αB-crystallin (αB), which have molecular weights of approximately 20 kDa each.[30–33] In vivo, bovine and calf lens α-crystallin form aggregates consisting of 30–50 subunits and have an average molecular weight of 800 kDa.[30,33–35] The aggregates have a ratio of 3αA:1αB for bovine lens α-crystallin.[30,36,37] Regini et al.[33] determined that 39 subunits form the bovine lens α-crystallin aggregate. Here, we will assume calf lens α-crystallin forms a 39 subunit aggregate for predicting the SASA of the monomer. Assuming calf lens α-crystallin contains aggregates of 39 subunits and no loss in surface area during aggregation, the SASA per subunit (20 kDa) is 10,710 ± 431 Å^2.

Babizhayev et al.[31,32] experimentally determined the hydration of young adult bovine lens α-crystallin to be approximately 1.9 g H_2O/g α-crystallin using the NMR spin-echo technique. This is in very good agreement with the regressed value of 1.433 ± 0.0409 g H_2O/g α-crystallin determined in this study. No ion binding studies, experimental or calculated, were found relative to α-crystallin for comparison.

Comparison of predicted SASA to molecular modeling estimates

X-ray diffraction results for the molecular structures of sheep methemoglobin (PDB: 2QU0[12]) and bovine β-lactoglobulin (PDB: 2Q2M,[14] 3NPO,[15] and 3BLG[16]) are deposited in the PDB. Thus, traditional computational modeling algorithms (Swiss-Pdb Viewer,[19] MOLMOL,[20] UCSF Chimera,[21] and GETAREA[22]) can be used to calculate and compare the respective SASA.

Using the highest quality option of Swiss-Pdb Viewer, the SASA for sheep methemoglobin was determined to be 24,304 Å^2. A high precision option (precision 6) in MOLMOL resulted in an estimated SASA for sheep methemoglobin of 24,981 Å^2. UCSF Chimera determined the SASA for sheep methemoglobin to be 26,100 Å^2. Finally, GETAREA calculated the SASA for sheep methemoglobin to be 24,759 Å^2. This range of values (24,304 Å^2-26,100 Å^2) is in excellent agreement with the free-solvent predicted value (22,398 ± 1,244 Å^2).

As noted above, three molecular structures (PDB: 2Q2M,[14] 3NPO,[15] and 3BLG[16]) were used to compute the SASA for bovine β-lactoglobulin. The values of the SASA for each of the three molecular structures for bovine β-lactoglobulin were calculated using the various computational software described above. Table 1 summarizes the range of the SASA for each structure. The range of SASA determined from these computational methods (7,500 Å^2-8,628 Å^2) was in good agreement with the SASA values determined from the free-solvent model (5,765 ± 1,031 Å^2 (pH 5.1), 6,656 ± 1,082 Å^2 (pH 6.0), and 9,141 ± 1,060 Å^2 (pH 8.0)). The minor potential pH dependency may be a result of the quality of the osmotic pressure data, particularly at near-saturation concentrations, where the free-solvent model is most sensitive.

Sensitivity of the free-solvent model hydration parameter for near-saturation concentrations

It has been previously shown that the free-solvent model regressed value of protein hydration from osmotic pressure data is sensitive to the protein concentration near its saturation limit.[5] Assuming a single protein in solution with a single monovalent salt. Then,

Table 1. Summary of the Regressed and Experimental Hydration Values and the Computational and Free-Solvent Model Predicted SASA

Macromolecule, MW (kDa)	Solution Properties, Salt Concentration, pH, Temperature	Regressed Hydration, ν_{12} $\frac{g\ H_2O}{g\ Protein}$	Literature Value of Hydration $\frac{g\ H_2O}{g\ Protein}$	PDB Code	Computational SASA (Å^2)	Free-Solvent Model SASA (Å^2)
β-Lactoglobulin (18.4)	0.1 M NaCl, 5.1, 20°C	0.809 ± 0.0138	0.72[29]	2Q2M[14]	7,724–8,628	5,765 ± 1,031
				3NPO[15]	7,666–8,644	
				3BLG[16]	7,500–8,304	
β-Lactoglobulin (18.4)	0.1 M NaCl, 6.0, 20°C	0.942 ± 0.0574	0.72[29]	2Q2M[14]	7,724–8,628	6,656 ± 1,082
				3NPO[15]	7,666–8,644	
				3BLG[16]	7,500–8,304	
β-Lactoglobulin (18.4)	0.1 M NaCl, 8.0, 20°C	1.313 ± 0.0206	0.72[29]	2Q2M[14]	7,724–8,628	9,141 ± 1,060
				3NPO[15]	7,666–8,644	
				3BLG[16]	7,500–8,304	
Hemoglobin (69)	0.1 M KCl, 7.43, 0°C	0.869 ± 0.0118	0.51[23,24]	2QU0[12]	24,304–26,100	22,398 ± 1,244
α-Crystallin (800)	0.15 M KCl, 6.8, room temperature	1.433 ± 0.0409	1.9[31,32]	N/A	N/A	417,691 ± 16,790

The computational SASA is reported for the minimum and maximum values determined from Swiss-Pdb Viewer (quality 6),[19] MOLMOL (precision 6),[20] UCSF Chimera,[21] and GETAREA.[22]

Table 2. Summary of the Regressed and Literature Ion Binding Values

Macromolecule, MW (kDa)	Solution pH	Salt Concentration	Regressed Protein-Ion Binding, v_{32} $\frac{\text{mol Salt}}{\text{mol Protein}}$	Literature Value of Protein-Ion Binding $\frac{\text{mol Salt}}{\text{mol Protein}}$
β-Lactoglobulin (18.4)	5.1	0.1 M NaCl	1.76 ± 0.068	N/A
β-Lactoglobulin (18.4)	6.0	0.1 M NaCl	1.17 ± 0.285	N/A
β-Lactoglobulin (18.4)	8.0	0.1 M NaCl	1.57 ± 0.102	N/A
Hemoglobin (69)	7.43	0.1 M KCl	5.20 ± 0.135	6 (pH 7.4)[25]
α-Crystallin (800)	6.8	0.15 M KCl	169 ± 5.27	N/A

$$\frac{\partial \pi}{\partial v_{12}} = \frac{RT}{\overline{V}_1}\left(\frac{x_2^{II}}{x_1^{II}} - \left(1 + \frac{v_{32}}{v_{12}}\right)x_2^{II}\right), \quad (2)$$

where x_i^{II} is the mole fraction of water ($i = 1$) and protein ($i = 2$) in the solution chamber of an osmometer, respectively. As can be seen, as $x_2^{II} \to 1$, $x_1^{II} \to 0$, resulting in a dramatic increase in sensitivity of the osmotic pressure prediction to the regressed hydration parameter, v_{12}. Thus, regression of near-saturation osmotic pressure data provides a more accurate estimate of the protein hydration parameter. This will, inevitably, lead to a more accurate prediction of the SASA.

From the literature values used in this study, the hemoglobin and α-crystallin osmotic pressure data was sufficient for predicting the SASA. However, the β-lactoglobulin data is limited at the near-saturation concentration range which may explain the variability in predicted SASA.

Moderate ionic strength

Yousef et al.[6] previously reported that moderate salt concentrations are required for the free-solvent model to accurately predict the osmotic pressure and regressed parameters. At low ionic strengths, such as 0.01 M, protein-protein interactions can occur, especially at near-saturation concentrations, because the Debye screening is reduced.[6] This consequence of low ionic strengths causes the free-solvent model, in its current form which does not account for aggregation, to have poor predictability.

Solutions which contain high ionic strengths can similarly result in poor prediction for the free-solvent model in its current form. High ionic strengths, such as 1 M, can affect the accuracy of the free-solvent model regressed hydration due to non-negligible competitive hydration of the salt ions.

For both low and high ionic strength solutions, the free-solvent model can be modified to account for protein–protein interaction (low ionic strength) or ion hydration (high ionic strength). This is a subject for our future work.

Conclusions

The free-solvent model is an appropriate representation of protein osmotic pressure in solution (albeit with moderate ionic strength[6]) and the free-solvent model analysis of concentrated protein solutions can be used as a tool to predict hydration and ion binding, and, most importantly, the SASA of the protein. The fact that the regressed hydration value for all globular proteins examined to date results in values corresponding to a monolayer, that the ion binding data is consistent with independently determined methods,[25–28] and that the resulting covariance for the regression of hydration and ion binding is extremely low, are compelling reasons in establishing credibility of the corresponding predictions from the free-solvent model. These observations collectively indicate that the model regressed parameters, when considering osmotic pressure data up to saturation, can be used to predict the SASA, hydration, and ion binding for soluble globular proteins. The SASA, hydration, and ion binding for sheep hemoglobin, bovine β-lactoglobulin, and calf lens α-crystallin were determined by regression of the free-solvent model for their osmotic pressure data found in the literature. The predicted SASA for sheep hemoglobin was in excellent agreement with the calculated value determined from the protein crystal structure. The predicted SASA for bovine β-lactoglobulin were in good agreement with the calculated values determined from the protein crystal structures when using osmotic pressure data previously reported in the literature. The predicted SASA for calf lens α-crystallin, which has no available protein structure, is reasonable in comparison with similar globular protein estimates.

Several critical applications of this method is that it provides an experimental alternative to estimating SASA, as well as changes in SASA upon formation of protein complexes. Currently, crystallographic or NMR structures of protein complexes are needed to perform this task. Solution structure determination of protein complexes by NMR is constrained by the molecular mass limitation of NMR spectroscopy. Furthermore, structure determination of protein complexes by crystallography is limited by the difficulty of determining the crystallization conditions. Additionally, crystal structures often suffer from crystal packing effects which may alter association interfaces or introduce nonspecific and nonphysiological interactions. The study of SASA is further exacerbated by the vast number of proteins (approximately 1 million[38]) and limited number of protein structures deposited into the PDB (approximately 66,000 as of February 2011). Extracting the SASA directly from experimental data of osmotic pressure may prove to be the more appropriate choice for these applications. Additionally, this method may prove to be advantageous in predicting the binding location of a drug or an inhibitor by determining the SASA which is at the binding interface using osmotic pressure data. Obtaining information on the SASA at the binding interface will provide a more direct way of determining possible binding locations compared with computational docking studies.

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Notation

N_i^k = initial number of moles of species i in compartment k
N^k = initial total number of moles in compartment k
R = ideal gas constant
T = temperature
$\overline{V}_i$ = specific volume of species i

Greek

v_{ij} = net number of moles of solvent component i interacting with protein j
π = osmotic pressure

Superscripts

I = compartment I (solvent)
II = compartment II (solution)

Subscripts

1 = solvent
2 = protein
3 = salt
i = individual species
j = individual protein species

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