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Separating Chemical and Excluded Volume Interactions of Polyethylene Glycols with Native Proteins: Comparison with PEG Effects on DNA Helix Formation

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Abstract

Small and large PEGs greatly increase chemical potentials of globular proteins (μ_2), thereby favoring precipitation, crystallization, and protein-protein interactions that reduce water-accessible protein surface and/or protein-PEG excluded volume. To determine individual contributions of PEG-protein chemical and excluded volume interactions to μ_2 as functions of PEG molality m_3 , we analyze published chemical potential increments $\mu_{23} = d\mu_2/dm_3$ quantifying unfavorable interactions of PEG (PEG200-PEG6000) with BSA and lysozyme. For both proteins, μ_{23} increases approximately linearly with the number of PEG residues (N_3). A 1 molal increase in concentration of PEG -CH₂OCH₂- groups, for any chain-length PEG, increases μ_{BSA} by ~2.7 kcal/mol and $\mu_{lysozyme}$ by ~1.0 kcal/mol. These values are similar to predicted chemical interactions of PEG -CH₂OCH₂- groups with these protein components (BSA ~3.3 kcal/mol, lysozyme ~0.7 kcal/mol), dominated by unfavorable interactions with amide and carboxylate oxygens and counterions. While these chemical effects should be dominant for small PEGs, larger PEGs are expected to exhibit unfavorable excluded volume interactions and reduced chemical interactions because of shielding of PEG residues in PEG flexible coils. We deduce that these excluded volume and chemical shielding contributions largely compensate, explaining why the dependence of μ_{23} on N_3 is similar for both small and large PEGs.

Introduction

Throughout his career Don Crothers was deeply interested the thermodynamics of interactions of biopolymers with water, solutes, salts and ligands, and the thermodynamic consequences of these interactions for biopolymer processes^{1,2}. In this article in memory of Don, we develop a thermodynamic analysis of the interactions of polyethylene glycols (PEG) with proteins relevant for analyses of PEG effects on protein processes. Polyethylene glycols (PEGs) are remarkable solutes, with many significant applications in biochemistry, structural biology and the biomedical sciences including precipitating and crystallizing proteins³⁻⁹, solubilizing aromatic hydrocarbons¹⁰, and as osmotic and/or excluded volume

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agents¹¹⁻¹⁷. Since both low and high molecular weight PEG can be effective protein precipitants, it is clear that chemical (preferential) as well as excluded volume interactions of PEGs with native proteins are typically unfavorable. Here we assess the individual contributions of chemical interactions and excluded volume effects to the increase in protein chemical potential (μ_{protein}) upon addition of either low or high molecular weight PEG that makes these oligomers and polymers very effective perturbants of protein processes including protein crystallization.

We analyze an extensive set of dialysis-densimetry data⁶ quantifying derivatives $d\mu_{\text{protein}}/dm_{\text{PEG}} = \mu_{23}$ quantifying effects of PEG concentration (PEG200 – PEG6000; component 3) on the chemical potentials of two proteins (component 2), bovine serum albumin (BSA) and lysozyme, which differ greatly in surface area and surface composition. Values of μ_{23} express the change in protein chemical potential (in kcal/mol) as the PEG concentration is increased by 1 molal; they are also equal to transfer free energies for the process of transferring the protein from a PEG-free solution to 1 molal PEG. Chemical interactions of any PEGs with these proteins are predicted using results of a recently-reported analysis of μ_{23} values for interactions of tetraEG and glycerol with model compounds¹⁸.

Our approach to separate chemical interactions and excluded volume effects of high molecular weight PEGs is similar to that used recently to separate these effects on melting of DNA hairpin helices and duplexes. Knowles et al.¹⁹ determined m -values (free energy derivatives $dG^{\circ}_{\text{obs}}/dm_3 = m\text{-value} = \mu_{23}$) at 313 K quantifying effects of molal concentration m_3 of ethylene glycol (EG) monomer and a series of PEGs from diEG to PEG20000 (number of monomer units $N_3 = 450$) on standard free energy changes ($G^{\circ}_{\text{obs}} = -RT\ln K_{\text{obs}}$) for melting a 12 bp duplex and a 12 base (4 bp) hairpin DNA oligomer. Values of μ_{23} obtained in this way are interpreted as the sum of two contributions: 1) helix-destabilizing, favorable preferential chemical interactions (PI) of the PEG with the primarily-nucleobase surface of DNA exposed in melting (μ_{23}^{pi}) and 2) the difference in DNA-PEG excluded volume (EV) interactions (μ_{23}^{ev}) between melted strand(s) and the helical form, favoring the latter. The analogous expression for μ_{23} is Eq. 1 (in Table 1 below).

Chemical interactions are deduced to be the primary contributor to DNA helix melting m -values for the smallest PEGs (PEG200). For large PEGs both excluded volume effects and chemical interactions are found to be important, though for large PEG flexible coils chemical interactions are reduced on a per-residue basis because of shielding of PEG residues in the interior of a flexible coil from interaction with DNA. Both chemical (μ_{23}^{pi}) and excluded volume (μ_{23}^{ev}) contributions to the PEG m -value (μ_{23}) are predicted to be proportional to PEG size (N_3), and indeed proportionality of the PEG m -value to N_3 is observed at both low and high N_3 , with very different proportionality constants in these two regimes of N_3 (¹⁹; also see Fig 4 below).

Here we apply a similar analysis to the extensive data set collected by Bhat and Timasheff⁶ quantifying effects of the PEG series PEG200-PEG6000 on chemical potentials of native lysozyme and BSA by dialysis and densimetry. These data, summarized in Fig. 1, reveal that

observed μ_{23} values are approximately proportional to N_3 over the full range of PEG sizes investigated (PEG 200–PEG 6000; $4 \leq N_3 \leq 136$). We predict chemical interaction contributions to the PEG-protein μ_{23} from PEG interior and end groups, and predict the excluded volume contribution to μ_{23} for large PEGs. Predictions based on net-unfavorable chemical interactions alone, with no shielding correction, are unexpectedly in semi-quantitative agreement with PEG-protein μ_{23} values, exhibiting near-proportionality of μ_{23} to N_3 for the full range of PEG sizes investigated. But unfavorable excluded volume effects (also proportional to N_3) are predicted to make significant contributions to these PEG-protein μ_{23} for the larger PEGs investigated. We conclude that for high- N_3 PEGs the unfavorable excluded volume contribution to μ_{23} must be masked by a reduction in the unfavorable chemical contribution to μ_{23} . We propose that this reduction occurs because of shielding of approximately quarter of the monomer residues of a PEG flexible coil from interactions with these proteins. In Discussion, we compare chemical interaction and excluded volume contributions to μ_{23} of PEG- folded protein interactions and to μ_{23} for PEG effects on DNA helix formation (changing PEG-nucleobase interactions) as a function of PEG size (N_3).

Results

Chemical Potential Derivatives Quantifying Interactions of Folded Proteins with PEGs

Bhat and Timasheff⁶ determined chemical potential derivatives $d\mu_{\text{protein}}/d\mu_{\text{PEG}} = \mu_{23}$ for interactions of the PEG series from PEG 200 to PEG 6000 (component 3) with BSA and lysozyme (component 2) at pH 7 by dialysis and densimetry. PEG interactions with BSA and three other proteins were also investigated at highly acidic pH, but these μ_{23} values appear challenging to interpret, perhaps because of conformational changes induced by the combination of acid pH and high PEG residue concentration.

PEG μ_{23} values for BSA and lysozyme (Fig. 1) are large and positive, showing that these interactions are highly unfavorable. Values of μ_{23} for both proteins increase approximately in proportion to PEG size (N_3). Slopes of best-fit lines through these data (with floated intercept) reveal that a 1 molal increase in concentration of PEG interior $-\text{CH}_2\text{OCH}_2-$ residues, added as *any* chain-length PEG in the range investigated, increases μ_{BSA} by ~ 2.7 kcal/mol and μ_{lysozyme} by ~ 1.0 kcal/mol. Compared for any size PEG, μ_{23} for PEG-BSA interaction is at least three-fold larger than μ_{23} for PEG-lysozyme interaction. Is this difference primarily the consequence of chemical interactions with the four-fold larger area and more anionic character of the BSA surface, and/or does it arise primarily from excluded volume interactions with the five-fold larger molecular volume of BSA?

Prediction of Chemical Contributions to μ_{23} from Interactions of PEG with Native Protein Surface and Counterions—Chemical contributions μ_{23}^{pi} to PEG-protein interactions are predicted using recently-determined α - and β -values quantifying the interactions of interior $(-\text{CH}_2\text{OCH}_2-)$ and two end $(-\text{CH}_2\text{OH})$ groups of PEG with unit area of each protein surface type and the salt ions of the *electroneutral* protein component (Eq. 2, Table 1;¹⁸), together with ASA information for each type of functional group on the surface

of these proteins. The α - and β -values needed for these calculations are summarized in Panels A and D of Fig 2, and ASA values are listed in Table S1.

For these predictions, we use the simplest model of the electroneutral protein component, in which only the number of salt ions needed to compensate the net charge on these proteins at pH 7 are included: $(\text{Na}^+)_{14}\text{BSA}^{14-}$ and $\text{lysozyme}^{9+} (\text{Cl,phosphate})^{9-}$. For lysozyme the anion calculation is approximate because of the uncertainty in how many of each anion are part of the electroneutral component and the unknown β -value for H_2PO_4^- . The details of the prediction made here are in Tables S1, S2. Other models of the salt ion composition of the electroneutral protein component have been considered but are more arbitrary and appear less useful.

Comparison of Panels A and D of Fig. 2 shows that α -values for interactions of PEG interior and end groups with most protein groups are either both favorable (aromatic C, cationic N and amide N groups) or both unfavorable (amide O, carboxylate O and hydroxyl O). β -values for interactions of both types of PEG groups with inorganic salt ions are also unfavorable. An exception is aliphatic C, for which α -values are of opposite sign for interactions with interior PEG groups (favorable) and end groups (unfavorable). α - and β -Values for interactions of protein groups and ions with two PEG end groups are generally weaker than the corresponding interactions with one PEG interior group.

Predicted contributions to μ_{23} from chemical interactions of PEG interior and end groups with functional groups (i) on these native protein surfaces ($\alpha_i\text{ASA}_i$) and counterions ($\beta_j v_j^{\text{ion}}$) are summarized in panels B, C, E, F of Fig 2 (see also Table S1). The dominant contributions are predicted to be unfavorable interactions of interior (and end) PEG groups with amide O, carboxylate O and salt counterions, counterbalanced to an extent by favorable interactions with C and N groups.

Net-unfavorable chemical interactions of native BSA and lysozyme (including the salt ions of the component) with PEG interior groups are predicted to increase the chemical potential of BSA by $3.31 \text{ kcal mol}^{-1}$ and that of lysozyme by $0.80 \text{ kcal mol}^{-1}$ for an increase in concentration of PEG interior groups of 1 molal (see Table 2). PEG end group interactions are predicted to be somewhat less unfavorable: $2.07 \text{ kcal mol}^{-1}$ for BSA and $0.57 \text{ kcal mol}^{-1}$ for lysozyme for an increase in concentration of PEG end groups of 1 molal. These overall predictions are shown as purple bars in Fig. 2 and listed in Table 2.

For sufficiently small PEGs, where chemical interactions entirely determine μ_{23} , interior group interactions should predict the slope of a plot of μ_{23} vs. $N_3 - 1$ and end group interactions should predict the intercept. Even though none of the PEGs investigated may be small enough for chemical interactions to be the only significant determinant of μ_{23} (¹⁹; also see Fig 4 and 5 below and accompanying text), Table 2 shows quite good agreement between predicted (above) and observed interior PEG group interactions with both BSA and lysozyme: 3.31 kcal/mol (predicted) vs. 3.6 kcal/mol (observed) for BSA; 0.80 kcal/mol (predicted) vs. 0.47 kcal/mol (observed) for lysozyme. “Observed” values are obtained from the slopes of plots of μ_{23} vs. N_3 for the four smallest PEGs investigated (PEG 200-PEG 1000) with intercepts fixed at the predicted PEG end group interaction (Fig 1; Table 2).

Scatter in these low- N_3 interaction data makes it impractical to float the intercepts in these fits. Similar estimates of observed PEG residue-protein interaction values are obtained from the slopes of plots of μ_{23} vs. N_3 for all eight PEGs investigated (PEG 200-PEG 6000): 2.7 kcal/mol (BSA) and 1.0 kcal/mol (lysozyme).

Predicted chemical contributions to protein interactions with any size PEG (before correction for shielding (χ) effects, if necessary) are:

$$\begin{array}{ll} \text{Lysozyme}^{9+}(\text{CI, phosphate})^{9-}: & \mu_{23}^{pi}=0.80 \quad (N_3 - 1) + 0.57 = 0.80 \quad N_3 - 0.23 \quad (\text{Eq.} \\ (Na^+)_{14}\text{BSA}^{14-}: & \mu_{23}^{pi}=3.31 \quad (N_3 - 1) + 2.07 = 3.31 \quad N_3 - 1.24 \quad 6) \end{array}$$

where the units of μ_{23}^{pi} are $\text{kcal mol}^{-1} \text{molal}^{-1}$. Uncertainties in these values are reported in Table 2. These predicted values of μ_{23}^{pi} for any size PEG are plotted vs $N_3 - 1$ in Fig. 3 as the red lines. Contributions of the constant terms in Eq. 6 (the predicted differences between interactions of internal and end PEG groups with these proteins) are small even for PEG 200 (tetraEG) and negligible (given the experimental uncertainty) for larger PEGs. Hence μ_{23}^{pi} is predicted to be approximately proportional to N_3 (also to $N_3 - 1$) for larger PEGs, as the red curves in Fig 3 indicate.

One interpretation of the finding that predicted μ_{23}^{pi} values for chemical PEG-protein interactions are in near-quantitative agreement with experimental results, not only for the smaller PEGs investigated but for larger PEGs as well, would be that neither chemical shielding (χ) effects nor excluded volume effects of large PEGs make significant contributions to the observed linear dependence of μ_{23} on N_3 . However, predictions of the excluded volume contribution indicate a different interpretation: excluded volume effects are significant for interactions of both proteins with larger N_3 PEGs, but the unfavorable contribution of excluded volume effects to μ_{23} is largely offset and therefore masked by a reduction in unfavorable chemical interactions because of shielding of interactions of PEG residues in the interior of a PEG flexible coil with these proteins. These predictions are developed in the following section.

Predicting Excluded Volume Contributions to μ_{23} for Interactions of Large PEGs with Proteins

Equations derived by Hermans²⁰ to predict the excluded volume between a flexible-coil polymer like high- N_3 PEG and a spherical protein or cylindrical DNA oligomer are summarized in Eqs. 3 and 4 (Table 1). These successfully predict the functional form and magnitude of the unfavorable (stabilizing) excluded volume contribution to PEG m -values for DNA oligomer melting, where m -value = μ_{23} (¹⁹; see Table 3 below) The key prediction of this analysis is that excluded volume contributions to the μ_{23} terms that contribute to PEG molal m -values are proportional to N_3 at high N_3 (Eqs. 3-4, Table 1). Using a statistical segment length (Kuhn length) $L_K = 12.6$ Å and modeling lysozyme and BSA as spheres of radii 15.8 Å and 27.0 Å, respectively, we predict the following excluded volume effects for high- N_3 PEGs (Table 2; see Supplemental):

$$\begin{array}{ll} \text{Lysozyme: } \mu_{23}^{ev} \approx 0.44 & N_3 \text{ kcal mol}^{-1} \text{ molal}^{-1} \\ \text{BSA: } \mu_{23}^{ev} \approx 0.75 & N_3 \text{ kcal mol}^{-1} \text{ molal}^{-1} \end{array} \quad \text{Eq 7}$$

Unfavorable excluded volume contributions μ_{23}^{ev} (Eq. 7), plotted in Figure 3 (purple lines), are approximately 25% (BSA) to 50% (lysozyme) as large as the corresponding chemical contributions μ_{23}^{pi} at any large N_3 .

The sum of chemical and excluded volume interactions $\mu_{23}^{pi} + \mu_{23}^{ev}$ (from eqs. 6 and 7), plotted as dotted lines in Fig 3, clearly predicts too unfavorable a PEG-protein interaction to be consistent with the experimental data. This lack of agreement between prediction and experiment for high- N_3 PEGs indicates that the predicted contribution of chemical interactions to μ_{23} for high- N_3 PEGs is too large. A reduction in this chemical contribution for high- N_3 PEGs was proposed previously¹⁹, based on the expectation that a fraction $(1-\chi)$ of PEG residues in the interior of PEG flexible coils are inaccessible to these proteins. This shielded fraction $(1-\chi)$ has not yet been investigated by theory or simulations, and its dependence on PEG N_3 and protein size is unknown. Here we treat it as a constant (χ_∞) to be determined by comparison of predicted and observed values of μ_{23} for high- N_3 PEG. From the DNA 12-mer analysis (Supplemental and Table 3 below), we find $\chi_\infty = 0.6 \pm 0.06$ (i.e. 40% of PEG residues are inaccessible to the 12-mer DNA oligomers). For lysozyme and BSA, a somewhat larger $\chi_\infty = 0.74$ provides a better fit to the experimental data, as summarized in Table 2. Fig. 3 plots $(1 - \chi_\infty) \mu_{23}^{pi}$, the reduction in μ_{23}^{pi} from this shielding effect, vs $N_3 - 1$ and shows that for both proteins this reduction in μ_{23}^{pi} is comparable in magnitude to μ_{23}^{ev} .

With $\chi = 0.74$ we obtain for high- N_3 PEGs:

$$\begin{array}{ll} \text{Lysozyme}^{9+} (CI, \text{phosphate})^{9-}: & \mu_{23} = \chi_\infty \mu_{23}^{pi} + \mu_{23}^{ev} = 1.03 \quad N_3 - 0.17 \\ (Na^+)_{14} \text{BSA}^{14-}: & \mu_{23} = \chi_\infty \mu_{23}^{pi} + \mu_{23}^{ev} = 3.2 \quad N_3 - 0.92 \end{array} \quad \text{Eq. 8}$$

where the units of μ_2 are $\text{kcal mol}^{-1} \text{ molal}^{-1}$. The intercepts in Eq. 8 are negligible for high- N_3 PEGs. If χ_∞ is independent of N_3 then Eq. 8 predicts that μ_{23} is proportional to N_3 for high- N_3 PEGs. These predicted μ_{23} values are plotted in Figure 3 (blue lines) and clearly are a reasonable fit to the high- N_3 PEG data, with a slope which is not very different from that of the low- N_3 PEG data.

For lysozyme, unfavorable chemical interactions of accessible PEG residues and excluded volume effects are each predicted to account for half of the unfavorable effect of high- N_3 PEGs on the chemical potential of BSA. The observed result is $\mu_{23} = 1.18 N_3$, which agrees with the prediction ($\mu_{23} = 1.03 N_3$) within 10-15% for this choice of χ_∞ .

For BSA unfavorable chemical interactions of accessible PEG residues are predicted to account for approximately two-thirds, and excluded volume one-third, of the PEG effect on the chemical potential of BSA. The experimental result is $\mu_{23} = 2.94 N_3$, in agreement with the prediction ($\mu_{23} = 3.2 N_3$) within 5-10% for this choice of χ_∞ .

We conclude that the thermodynamic consequences for PEG-protein interactions of linking PEG residues together to form a high- N_3 chain polymer (excluded volume and shielding effects of PEG flexible coils) are largely compensating at the level of μ_{23} .

Discussion

Comparisons of Chemical, Excluded Volume Contributions to PEG-Protein Interactions and to PEG Effects on Duplex and Hairpin DNA Melting: Determining When Excluded Volume Effects are Most Important

Here we compare results and conclusions of the present study of chemical and excluded volume contributions to PEG-native protein interactions with previous analysis of chemical and excluded volume contributions to PEG m -values for melting of duplex and hairpin DNA oligomer helices. PEG m -values for oligomer DNA melting are equal to differences in μ_{23} between the melted strand(s) and the helix (m -value = μ_{23} , where from Eq. 5 $\mu_{23} = \chi\mu_{23}^{\text{pi}} + \mu_{23}^{\text{ev}}$). This parallels the analysis of PEG-native protein interactions, quantified by values of μ_{23} where $\mu_{23} = \chi\mu_{23}^{\text{pi}} + \mu_{23}$. In both cases, the shielding factor $\chi \approx 1$ and $\mu_{23}^{\text{ev}} \approx 0$ for sufficiently small- N_3 PEGs, and $\chi = \chi_{\infty}$ for high- N_3 PEGs. (For simplicity, the same χ_{∞} is used for helix and melted strands.)

The chemical term μ_{23}^{pi} for DNA melting is interpreted by analogy with Eq. 2 as the sum of interactions of the interior and end groups of PEG with the nucleobase ASA exposed in melting¹⁹. The excluded volume term μ_{23}^{ev} is the difference in excluded volume interactions with the melted strand(s) and the double helix; this term is particularly significant for duplex melting where two strands are formed from one.

Experimentally, μ_{23} values for PEG-native protein interactions are linear functions of N_3 (approximately proportional to N_3) for both low- and high- N_3 PEGs, with positive slopes that are sufficiently similar that all eight PEG μ_{23} values for each protein can be fit by a single line over the range of N_3 investigated (Fig. 1). Predictions of μ_{23}^{pi} and μ_{23}^{ev} in table 2 and Fig. 3 show that both are proportional to N_3 at high N_3 , and that μ_{23}^{pi} is a linear function of (nearly proportional to) N_3 at low N_3 . Both μ_{23}^{pi} and μ_{23}^{ev} are positive (unfavorable).

Paralleling this behavior of PEG-protein interactions, panels B and C of Fig. 4 show that PEG m -values = μ_{23} for duplex and hairpin melting also are linear functions of N_3 (approximately proportional to N_3) for both low- and high- N_3 PEGs. (These experimental data were originally reported as monomolal PEG m -values (i.e. μ_{23}/N_3), which to a good approximation are independent of N_3 at both small and large N_3 (see Figure 4A).)

Comparison of Fig 4 B, C with Fig. 1 shows a key difference between the low- N_3 PEG-protein and PEG-DNA data. PEG-DNA μ_{23} values are negative for EG, and become increasing negative with increasing N_3 between EG and tetraEG by $-0.32 \text{ kcal mol}^{-1} (\text{molal PEG residues})^{-1}$ for duplex and $-0.16 \text{ kcal mol}^{-1} (\text{molal PEG residues})^{-1}$ for hairpin.

Therefore chemical interactions between PEG residues and the DNA nucleobase surface exposed in melting are favorable, while chemical interactions of PEGs with native BSA and lysozyme are unfavorable (Fig.2, Table 2). The two-fold difference in PEG effects for 12-bp duplex vs 12-mer hairpin melting is consistent with the ratio of ASA of melting for duplex and hairpin, and supports the interpretation of these effects as purely chemical interactions.

In the vicinity of tetraEG, PEG-DNA μ_{23} values exhibit a minimum for duplex DNA melting and increase for larger- N_3 PEGs, becoming positive and again approximately proportional to N_3 for $N_3 > 10$. For hairpin melting, PEG-DNA μ_{23} values decrease less rapidly with increasing N_3 above tetraEG. PEG effects on duplex and hairpin melting in these low- and high- N_3 regions are summarized in Table 3. Since the smallest PEG studied with native BSA and lysozyme was PEG200 (tetraEG), it is possible that these PEG effects are not purely from chemical interactions.

Predictions of excluded volume contributions (μ_{23}^{ev}) of high N_3 PEGs on duplex and hairpin melting in Table 3 and Fig. 5 A,B (analogous to Fig 3) show that these quantities are positive (unfavorable; helix stabilizing) and proportional to N_3 . The predicted excluded volume effect is much larger for duplex melting ($0.34 \text{ kcal mol}^{-1} (\text{molal PEG residues})^{-1}$) than for hairpin melting ($0.058 \text{ kcal mol}^{-1} (\text{molal PEG residues})^{-1}$) primarily because of the stoichiometry of duplex melting, forming two strands from one duplex.

The behavior of μ_{23} for DNA melting as a function of PEG N_3 at high PEG N_3 predicted from $\mu_{23}^{pi} + \mu_{23}^{ev}$ (dotted lines in Fig 4 D, E) agrees qualitatively but not quantitatively with the observed μ_{23} . We attributed the difference to shielding of a fraction ($1 - \chi_\infty$) of PEG residues in the interior of PEG flexible coils, reducing the per-residue interaction of PEG with DNA¹⁹. Table 3 and Fig 5 A, B show that quantitative agreement is obtained for $\chi_\infty \approx 0.6$ (blue lines on Fig. 5), similar to the best fit value for PEG-protein interactions ($\chi_\infty \approx 0.74$).

Comparison with Other Interpretations of PEG Interactions, PEG Effects

Previous analyses of PEG interactions and/or PEG effects on protein or nucleic acid processes have generally interpreted these either as primarily excluded volume effects, as the complete-exclusion (osmotic) limit of a chemical effect, or by other mechanisms^{5,6,13,21-27}. The PEG-protein interactions summarized in Figure 1 were originally interpreted as a steric (excluded volume) effects⁶. An insightful review pointed out that PEG-protein interaction and PEG effects on protein processes should not be interpreted simply as excluded volume effects because of its chemical interactions with proteins¹³. Here we separate and quantify these effects based on additivity (Eq. 1). Some *previous analyses* of chemical interaction and excluded volume effects of solutes on biopolymer conformational transitions (protein folding) have also been based on additivity of these contributions to the m-value (μ_{23}). In these studies, chemical interactions of the solute with the protein were evaluated empirically from experimental m-values and predictions of solute-protein excluded volume contributions²⁸⁻³⁰. A recent detailed experimental study of polyol m-values for unfolding of a beta-hairpin found large, compensating enthalpic and entropic contributions to the smaller-magnitude free energy m-value, consistent with a major role of chemical interactions in determining these m-values³¹⁻³³. Here we determine chemical interactions from small compound data¹⁸, and calculate excluded volume interactions of large PEGs with proteins and nucleic acids using Hermans' theory²⁰.

The analysis of effects of the entire PEG series on DNA helix formation (Fig 5; see also Knowles et al¹⁹) shows that both chemical and excluded volume contributions are

significant for larger PEG oligomers ($N_3 > 4$) and polymers, especially where the process involves a change in molecularity (e.g. duplex to 2 ss). Where only a change in shape is involved, as for hairpin DNA melting, we find (and predict) that excluded volume effects are much smaller in magnitude.

We conclude that chemical interactions of PEGs with proteins and nucleic acids make significant contributions to μ_{23} or μ_{23} (i.e. m-values) for all PEG sizes. Knowles et al.¹⁹ deduced that chemical interactions are the only significant contributor to m-values for effects of tetra EG or smaller PEGs on nucleic acid helix formation. Knowles et al.¹⁸ demonstrated that an interpretation based entirely on chemical interactions is sufficient to interpret μ_{23} values for the interactions of tetraEG and glycerol with a variety of small model compounds. These findings are consistent with previous analyses of the effects of urea on nucleic acid helix formation³⁴, and effects of urea, glycine betaine, proline and other small solutes on protein folding³⁵⁻³⁸. Likewise an interpretation based entirely on chemical interactions is sufficient to interpret μ_{23} values for the interactions of urea, proline and glycine betaine with a variety of small model compounds^{34,36-38}.

Chemical interactions play a major role in both PEG-native protein interactions and PEG effects on DNA melting for even the largest PEGs investigated (PEG20000 in DNA melting studies, PEG6000 in studies with native proteins). These chemical interactions differ greatly from favorable to unfavorable for different functional groups. For both native proteins and the DNA melting processes studied, we deduce that chemical interactions per PEG residue are reduced for high- N_3 PEGs by shielding of a moderate fraction of PEG residues (one-quarter and one-third for these examples), presumably those deep in the interior of the PEG flexible coil. Both chemical and excluded volume effects of PEG are found to be proportional to PEG size (N_3) for high- N_3 PEGs, and chemical effects of PEG are also proportional to N_3 for low- N_3 PEGs. The analysis developed and applied in this and two previous papers^{18,19} provides a general method to separate and interpret chemical (PI, χ) and excluded volume interactions of all PEGs with biopolymers, as well as chemical and excluded volume effects of all PEGs on any biopolymer process.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

PEG Size Dependence of Unfavorable PEG-BSA and PEG-Lysozyme Interactions. Values of the chemical potential derivative $d\mu_2/dm_3$ (μ_{23} , in kcal/mol per molal of PEG molecules) for interactions of PEGs with native BSA (diamonds) and lysozyme (triangles) at pH 7, plotted vs. the degree of polymerization N_3 . Solid lines are linear least squares fits of all 8 points (PEG 200- PEG 6000) with floated intercept. Dashed lines are fits to the higher- N_3 PEG data (PEG 2000, 3000, 4000, and 6000) with floated intercept. Dotted lines are fits to the lower- N_3 PEG data (PEG 200, 400, 600, 1000) with intercept fixed at the predicted value for two PEG end groups. Slopes and intercepts of solid lines for BSA with the uncertainty as 1 standard deviation (2.70 ± 0.13 , 8.71 ± 8.6) and Lysozyme (1.00 ± 0.089 , -9.24 ± 5.8); of dashed lines for BSA (2.94 ± 0.34 , -15.3 ± 32) and Lysozyme (1.18 ± 0.24 , -26.9 ± 21); of dotted lines for BSA (3.62 ± 0.36 , -1.40) and Lysozyme (0.47 ± 0.046 , 0.10). Intercepts for dotted lines are fixed and therefore are reported without uncertainties.

Fig 2.

Prediction of Chemical (Preferential) Interactions of PEG Interior and End Groups with Functional Groups of Native BSA and Lysozyme. Panels A, D: Bar graph summary of interaction potentials for PEG interior group (A) and end group (D) with functional groups of protein surfaces (α values) and inorganic ions of protein component (β values; divided by 100 to put on same scale as alpha values), determined by Knowles, Shkel et al¹⁸. Other panels: Predicted contributions to μ_{23} from interactions of PEG interior repeat group and end group with functional groups of BSA (left side, panels B, E) and lysozyme (left side, panels C, F) and salt ions of their minimal electroneutral components (central portion of each

panel). Purple bars (right side of each panel) show predicted values of μ_{23}^{pi} from the sum of these protein and ion contributions.

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Figure 3.

Dissection of Unfavorable PEG-Protein Interactions into Contributions from Chemical Interactions, Shielding, and Excluded Volume Effects. Panel A : PEG-BSA interactions. Panel B; PEG-lysozyme interactions. Data points are the same as in Fig. 1 plotted vs N_3-1 . Red lines are predicted contributions of unshielded preferential (chemical) interactions (PI) to μ_{23} from Eq. (2) using α - and β -values from Fig. 2¹⁸ and ASA information from Table S1. Purple lines are predicted excluded volume (EV) contributions to μ_{23} of higher- N_3 PEGs (PEG2000). Green lines are reduction due to shielding of chemical (preferential) interactions ($(1-\chi)PI$) for a residue-accessibility factor $\chi = 0.74$ (see Table 2). Blue lines are predicted combinations of shielded chemical and excluded volume contributions ($\chi PI + EV$) to μ_{23} for higher- N_3 PEGs (PEG2000). Dashed black lines are sums of excluded volume (purple) and unshielded chemical (red) contributions (PI+EV).

Fig 4.

Analysis of m -Values Quantifying Effects of the Monomer-Polymer Series of PEGs (EG to PEG20000) on Stability of DNA Helices¹⁹. Panel A: Plot of monomolal PEG m -values (i.e. $m\text{-value}/N_3 = \Delta\mu_{23}/N_3$) vs. $\log N_3$. Panel B: Plot of molal scale PEG m -values ($\Delta\mu_{23}$) vs. number of interior PEG repeats ($N_3 - 1$) for small and intermediate PEGs ($N_3 \leq 14$). Lines for $N_3 \leq 3$ are linear fits of EG, diEG and triEG m -values. Lines for $N_3 > 13$ are from Panel C. Panel C: Plot of molal scale PEG m -values ($\Delta\mu_{23}$) vs. number of interior PEG repeats ($N_3 - 1$) for all ($N_3 - 1$) investigated. Lines are best fits to four (duplex) and five (hairpin) highest- N_3 PEG m -values with floated intercepts.

Figure 5.

Dissection of PEG-DNA Interactions into Contributions from Chemical Interactions, Shielding, and Excluded Volume Effects. Panel A : PEG-Duplex m -values (μ_{23}) . Panel B; PEG-Hairpin m -values (μ_{23}). Red lines are predicted contributions of unshielded preferential (chemical) interactions (PI) to μ_{23} calculated using observed lower- N_3 (EG-triEG) slopes from Figure 4B. Purple lines are predicted excluded volume (EV) contributions to μ_{23} of higher- N_3 PEGs (PEG1450). Green lines are reduction due to shielding of chemical (preferential) interaction contributions ((1- χ)PI) for a residue-accessibility factor $\chi = 0.6$ (see Table 3). Blue lines are predicted combinations of shielded chemical and excluded volume contributions (χ PI+EV) to μ_{23} for higher- N_3 PEGs (PEG1450). Dashed black lines are sums of excluded volume (purple) and unshielded chemical (red) contributions (PI+EV).

Table 1

Predictions of Chemical and Excluded Volume Contributions to PEG-Protein Interactions as a Function of PEG Size

Observable: Chemical potential derivative μ_{23} quantifying PEG-protein interaction ^a	$\mu_{23} = \left(\frac{\mu_2}{m_3} \right)_{P.T} = \chi \mu_{23}^{pi} + \mu_{23}^{ev}$	Eq.1
Chemical contribution μ_{23}^{pi} from PEG- protein and PEG-ion interactions ^b	$\mu_{23}^{pi} = \sum_i \alpha_{2E,i} ASA_i^{protein} + \sum_k \beta_{2E,k} v_k^{ion} + (N_3 - 1) \sum_i \alpha_{I,i} ASA_i^{protein} + (N_3 - 1) \sum_k \beta_{I,k} v_k^{ion}$	Eq.2
Excluded volume contribution $\mu_{23}^{ev,\infty}$ (large N_3 limit) ^c	$\mu_{23}^{ev,\infty} = RT \rho_1^* V_{23}^{ev}$	Eq.3
PEG- biopolymer excluded volume ^d (spherical proteins, DNA cylinders)	$V_{23}^{ev} = k L \frac{2}{K} N_K = k l L_K N_3 \text{ where } k_{sphere} = r_2 \frac{5\pi}{0}; k_{cylinder} = r_2 \frac{2.5\pi}{6} \frac{p}{C(p)}$	Eq.4
Combined chemical, χ and excluded volume contributions to μ_{23} in large N_3 limit	$\mu_{23}^{\infty} = \chi \mu_{23}^{pi} + \mu_{23}^{ev,\infty}$	Eq.5

^a In Eq.1, μ_{23}^{pi} and μ_{23}^{ev} are the chemical (preferential interaction) and excluded volume contributions to μ_{23} , and χ is the average fraction of residues of a PEG chain molecule that are accessible to the protein.

^b In Eq.2, α , β are interaction potentials for the interaction of the indicated PEG group (two $-\text{CH}_2\text{OH}$ ends (α_{2E}); interior $-\text{CH}_2\text{OCH}_2-$ (α_I)) with unit area of protein functional group i (see Figure 3 and ref.[18]) and with salt ions of the protein component (β_{2E} , β_I)

^c In Eq. 3, V_{23}^{ev} is the protein-PEG excluded volume (see Supplemental Materials).

^d In Eq. 4 [20], L_K is PEG Kuhn length and N_K is the number of Kuhn lengths per PEG molecule (see Supplemental Materials); $N_K L_K = N_3 l$ where l the length of a PEG residue (3.55 Å for $-\text{CH}_2\text{OCH}_2-$). Expressions for the geometrical quantity k are given for spheres and cylinders [20]: r_2 is sphere or cylinder radius, $p=L_2/r_2$ is the ratio of the length L_2 to the radius of the cylinder (the aspect ratio) and for the cylinder, function $C(p)$ is given in Supplemental Materials. Numerical factors in k are obtained from simulations [20].

Table 2

Observed and Predicted Contributions of Chemical (Preferential) Interactions (PI) and Excluded Volume (EV) to PEG-Native Protein Interactions at 25 °C ^a Units of all entries are kcal mol⁻¹(molal PEG residues)⁻¹

Interactions of Small PEGs with:	BSA ^b	Lysozyme ^c
Chemical: Contribution of a PEG Interior Group		
Observed ^d	3.62 ± 0.36	0.47 ± 0.05
Predicted ^e	3.31 ± 0.23	0.80 ± 0.07 ^l
Chemical: Contribution of 2 PEG End Groups ^f		
Predicted ^g	2.07 ± 0.34	0.57 ± 0.09 ^l
Interactions of Large PEGs with:		
	BSA ^b	Lysozyme ^c
Excluded Volume, Shielded Chemical Contributions Per PEG Residue		
Predicted excluded volume contribution ^h	0.75 ± 0.08	0.44 ± 0.04
Predicted reduction in chemical contribution from shielding ⁱ	-0.86 ± 0.125	-0.21 ± 0.06 ^l
Comparison of Observed and Predicted μ ₂₃ Per PEG Residue		
Observed ^j	2.94 ± 0.34	1.18 ± 0.24
Predicted ^k	3.2 ± 0.2	1.03 ± 0.07 ^l

^aUncertainty calculated as 1 standard deviation for linear least square fitting or propagated as described in [18]

^bNa₁₄BSA¹⁴⁻

^cLysozyme⁹⁺(Cl, phosphate)⁹⁻

^dSlope dμ₂₃/dN₃ of line fitted through the first 4 points (PEG 200- PEG 1000) of Fig 1 using predicted end-group contribution (see table) as the intercept.

^eSlope dμ₂₃^{pi}/dN₃ of red line in Fig.3, predicted from α_I, β_I, and ASA (Fig 2 A-C).

^fLow N₃ data are insufficiently accurate to obtain this from their intercept in Fig. 1 (not shown).

^gIntercept of red line in Fig. 3, predicted from α_I, β_I, and ASA (Fig 2 D-F)

^hPredicted from Eqs. 3-4 (Table 1) for BSA radius 27 Å, Lysozyme radius 15.8 Å.

ⁱCalculated as $-(1 - \chi_{\infty}) \mu_{23}^{pi} / N_3$ with $\chi_{\infty}=0.74$ (see text)

^jSlope dμ₂₃/dN₃ of dashed line on Fig. 1 (fitted through last 4 points, PEG 2000 – PEG 6000).

^k $(\chi_{\infty} \mu_{23}^{pi} + \mu_{23}^{ev}) / N_3$ predicted as in footnotes g and h with $\chi_{\infty}=0.74$. Corresponds to slope of blue line of Fig.3.

^lPredicted values calculated assuming the protein component is Lysozyme⁹(Cl)-9 (see Tables S1, S2) are 0.59 (instead of 0.80), 0.52 (instead of 0.57), -0.19 (instead of -0.21), and 0.92 (instead of 1.03) with error reduced proportionally.

Table 3

Contributions from Chemical (Preferential) Interactions (PI) and Excluded Volume (EV) to PEG Effects on DNA Duplex and Hairpin Melting. Units of all entries are kcal mol⁻¹molal PEG residues)⁻¹

	12 bp Duplex ^{a, b}	4 bp Hairpin ^{a, b}
Observed PEG-DNA Chemical Interaction Per PEG Residue^c	-0.322 ± 0.02	-0.160 ± 0.02
Predicted excluded volume effect Per PEG Residue^d	0.34 ± 0.03	0.058 ± 0.006
Predicted reduction in PEG-DNA chemical interaction from shielding^e	0.129 ± 0.02	0.064 ± 0.01
Observed, Predicted PEG Effects Per PEG Residue		
Observed^f	0.168 ± 0.01	-0.033 ± 0.003
Predicted^g	0.164 ± 0.037	-0.0346 ± 0.013

^aData obtained at 40 °C.

^bUncertainty calculated as 1 standard deviation for linear least square fitting or propagated as described in [18]

^cSlope d μ_{23}/dN_3 of line fitted through the first 3 points (EG - triEG) of Fig 4B and red lines of Fig.5.

^dPredicted from Eqs. 3-4 (Table 1) using DNA and PEG structural information in Table S3.

^eCalculated as $-(1 - \chi_{\infty}) \Delta\mu_{23}^{pi}/N_3$ with $\chi_{\infty}=0.6$ (see text)

^fSlope d μ_{23}/dN_3 of dashed line on Fig. 4C (fitted through last 4 points, PEG 2000 – PEG20000, for duplex and last 5 points, PEG 1450 – PEG20000, for hairpin).

^g $(\chi_{\infty} \Delta\mu_{23}^{pi} + \Delta\mu_{23}^{ev}) / N_3$ predicted as in footnotes d and e with $\chi_{\infty}=0.6$. Corresponds to slope of blue line of Fig.5.