ABSTRACT:
Small and large PEGs greatly increase chemical potentials of globular proteins ($\mu_2$), 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 $\mu_2$ as functions of PEG molality $m_3$, we analyze published chemical potential increments $\mu_23$ quantifying unfavorable interactions of PEG (PEG200–PEG6000) with BSA and lysozyme. For both proteins, $\mu_23$ increases approximately linearly with the number of PEG residues ($N_3$). A 1 molal increase in concentration of PEG -CH$_2$OCH$_2$- groups, for any chain-length PEG, increases $\mu_{\text{BSA}}$ by $\sim 2.7$ kcal/mol and $\mu_{\text{lysozyme}}$ by $\sim 1.0$ kcal/mol. These values are similar to predicted chemical interactions of PEG -CH$_2$OCH$_2$- groups with these protein components (BSA $\sim 3.3$ kcal/mol, lysozyme $\sim 0.7$ 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 $\mu_23$ on $N_3$ is similar for both small and large PEGs. © 2015 Wiley Periodicals, Inc. Biopolymers 103: 517–527, 2015.
Keywords: m-value; preferential interactions; PEG; polymer excluded volume

INTRODUCTION
Throughout his career, Don Crothers was deeply interested in the thermodynamics of interactions of biopolymers with water, solutes, salts and ligands, and the thermodynamic consequences of these interactions for biopolymer processes. 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 proteins, solubilizing aromatic hydrocarbons, and as osmotic and/or excluded volume agents. 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 ($\mu_{\text{protein}}$) 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 data quantifying derivatives $d\mu_{\text{protein}}/d\text{PEG} = \mu_{23}$ 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 $\mu_{23}$ 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 $\mu_{23}$ values for interactions of tetraEG and glycerol with model compounds.\textsuperscript{18}

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.\textsuperscript{19} determined $m$-values (free energy derivatives $d\Delta G_{\text{obs}}/dm = m$-value = $\Delta\mu_{23}$) 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 ($\Delta G_{\text{obs}} = -RT\ln K_{\text{obs}}$) for melting a 12 bp duplex and a 12 base (4 bp) hairpin DNA oligomer. Values of $\Delta\mu_{23}$ 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 ($\Delta\mu_{23}^{\text{PI}}$) and (2) the difference in DNA-PEG excluded volume (EV) interactions ($\Delta\mu_{23}^{\text{EV}}$) between melted strand(s) and the helical form, favoring the latter. The analogous expression for $\mu_{23}$ is Eq. (1) (in Table I below).

Chemical interactions are deduced to be the primary contributor to DNA helix melting $m$-values for the smallest PEGs ($\leq$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 ($\Delta\mu_{23}^{\text{PI}}$) and excluded volume ($\Delta\mu_{23}^{\text{EV}}$) contributions to the PEG $m$-value ($\Delta\mu_{23}$) 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 $N_3$ (Ref. [19]; also see Figure 4 below).

Here we apply a similar analysis to the extensive data set collected by Bhat and Timasheff\textsuperscript{8} quantifying effects of the PEG series PEG200-PEG6000 on chemical potentials of native lysozyme and BSA by dialysis and densimetry. These data, summarized in Figure 1, reveal that observed $\mu_{23}$ 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 $\mu_{23}$ from PEG interior and end groups, and predict the excluded volume contribution to $\mu_{23}$ for large PEGs. Predictions based on net-unfavorable chemical interactions alone, with no shielding correction, are unexpectedly in semi-quantitative agreement with PEG-protein $\mu_{23}$ values, exhibiting near-proportionality of $\mu_{23}$ 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 $\mu_{23}$ for the larger PEGs investigated. We conclude that for high-$N_3$, PEGs the unfavorable excluded volume contribution to $\mu_{23}$ must be masked by a reduction in the unfavorable chemical contribution to $\mu_{23}$. We propose that this reduction occurs because of shielding of approximately 0.25 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 $\mu_{23}$ of PEG folded protein interactions and to $\Delta\mu_{23}$ 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 Timasheff\textsuperscript{8} determined chemical potential derivatives $d\mu_{\text{protein}}/d\text{PEG} = \mu_{23}$ 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 $\mu_{23}$ values appear challenging to interpret, perhaps because of conformational changes induced by the combination of acid pH and high PEG residue concentration.

PEG $\mu_{23}$ values for BSA and lysozyme (Figure 1) are large and positive, showing that these interactions are highly unfavorable. Values of $\mu_{23}$ 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 -CH$_2$OCH$_2$- residues, added...
Table I Predictions of Chemical and Excluded Volume Contributions to PEG-Protein Interactions as a Function of PEG Size

<table>
<thead>
<tr>
<th>Observable: chemical potential derivative $\mu_{23}$ quantifying PEG-protein interaction$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_{23} = \left( \frac{\partial \mu_{23}}{\partial \mu_{12}} \right)<em>{P,T} = \mu</em>{23}^{pl} + \mu_{23}^{ev}$ Eq. (1)</td>
</tr>
</tbody>
</table>

- $\mu_{23}^{pl}$ from PEG-protein and PEG-ion interactions$^b$  
  
  $\mu_{23}^{pl} = \sum_i \alpha_{2E,i} \text{ASA}_{i}^{protein} + \sum_k \beta_{2E,k} \nu_{E_k}^{ion}$  
  + $(N_1 - 1) \sum_i \alpha_{E,i} \text{ASA}_{i}^{protein} + (N_3 - 1) \sum_k \beta_{E,k} \nu_{E_k}^{ion}$ Eq. (2)

- Excluded volume contribution $\mu_{23}^{ev}$ in large $N_3$ limit$^c$  
  
  $\mu_{23}^{ev} = RT \frac{1}{V_{23}^{ev}}$ Eq. (3)

- PEG-biopolymer excluded volume (spherical proteins, DNA cylinders)$^d$  
  
  $V_{23}^{ev} = kL_k^2 N_k = kL_k N_k$ Eq. (4)

  
  where $k_{sphere} = \frac{5\pi}{2} \frac{5}{2}$; $k_{cylinder} = \frac{2\pi r_a}{9}$

- Combined chemical, $\chi$ and excluded volume contributions to $\mu_{23}$ in large $N_3$ limit  
  
  $\mu_{23}^{\chi, ev} = \chi_{\chi, ev} \mu_{23}^{pl} + \mu_{23}^{ev}$ Eq. (5)

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

$^b$ In Eq. (2), $\alpha$, $\beta$ are interaction potentials for the interaction of the indicated PEG group (two –CH$_2$OH ends (\(\alpha_{2E}\)); interior –CH$_2$OCH$_2$ – (\(\alpha_{E}\))) with unit area of protein functional group $i$ (see Figure 2 and Ref. 18) and with salt ions of the protein component (\(\beta_{2E,k}\), $\beta_{E,k}$).

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

$^d$ In Eq. (4), $L_k$ is PEG Kuhn length and $N_k$ is the number of Kuhn lengths per PEG molecule (see Supporting Information); $k_{sphere} = \frac{5\pi}{2} \frac{5}{2}$; $k_{cylinder} = \frac{2\pi r_a}{9}$.

as any chain-length PEG in the range investigated, increases $\mu_{BSA}$ by $\sim 2.7$ kcal/mol and $\mu_{lysozyme}$ by $\sim 1.0$ kcal/mol. Compared for any size PEG, $\mu_{23}$ for PEG-BSA interaction is at least three-fold larger than $\mu_{23}$ 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 $\mu_{23}$ from Interactions of PEG with Native Protein Surface and Counterions

Chemical contributions $\mu_{23}^{pl}$ to PEG-protein interactions are predicted using recently-determined $\alpha$- and $\beta$-values quantifying the interactions of interior (-CH$_2$OCH$_2$) and two end (-CH$_2$OH) groups of PEG with unit area of each protein surface type and the salt ions of the electroneutral protein component [Eq. (2), Table I; Ref. 18, together with ASA information for each type of functional group on the surface of these proteins. The $\alpha$- and $\beta$-values needed for these calculations are summarized in Panels A and D of Figure 2, and ASA values are listed in Supporting Information Table SI.

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: (Na$^+$)$_{14}$BSA$^{14-}$ and lysozyme$^{9-}$ (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 $\beta$-value for H$_2$PO$_4^-$. The details of the prediction made here are in Supporting Information Tables SI and SII. 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 Figure 2 shows that $\alpha$-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 χ-values are of opposite sign for interactions with PEG interior groups (favorable) and end groups (unfavorable). χ- and β-values for interactions of protein groups and ions with two PEG end groups are generally weaker that the corresponding interactions with one PEG interior group.

Predicted contributions to \( \mu_{23} \) from chemical interactions of PEG interior and end groups with functional groups (i) on these native protein surfaces (\( \chi_{ASA_i} \)) and counterions (\( \beta_{1Y_j}^{\text{ion}} \)) are summarized in panels B, C, E, F of Figure 2 (see also Supporting Information Table SI). 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 kcal mol\(^{-1}\) and that of lysozyme by 0.80 kcal mol\(^{-1}\) for an increase in concentration of PEG interior groups of 1 molal (Table II). PEG end group interactions are predicted to be somewhat less unfavorable: 2.07 kcal mol\(^{-1}\) for BSA and 0.57 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 Figure 2 and listed in Table II.

For sufficiently small PEGs, where chemical interactions entirely determine \( \mu_{23} \), interior group interactions should predict the slope of a plot of \( \mu_{23} \) 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 \( \mu_{23} \) (Ref. 19; also see Figures 4 and 5 below and accompanying text), Table II 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 \( \mu_{23} \) vs. \( N_3 \) for the four smallest PEGs investigated (PEG 200-PEG 1000) with intercepts fixed at the predicted PEG end group interaction (Figure 1; Table II). Scatter in these low-N3 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 \( \mu_{23} \) 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 (\( \gamma \)) effects, if necessary] are:

Lysozyme\(^{+} \) (Cl, phosphate)\(^{-} \): \( \mu_{23}^{\text{pl}} = 0.80(N_3 - 1) + 0.57 = 0.80N_3 - 0.23 \) \( (\text{Na}^+)_{14}\text{BSA}^{14-} \): \( \mu_{23}^{\text{pl}} = 3.31(N_3 - 1) + 2.07 = 3.31N_3 - 1.24 \) \( \text{(6)} \)

where the units of \( \mu_{23}^{\text{pl}} \) are kcal mol\(^{-1}\) molal\(^{-1}\). Uncertainties in these values are reported in Table II. These predicted values of \( \mu_{23}^{\text{pl}} \) for any size PEG are plotted vs. \( N_3 - 1 \) in Figure 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 \( \mu_{23}^{\text{pl}} \) is predicted to be approximately proportional to \( N_3 \) (also to \( N_3 - 1 \)) for larger PEGs, as the red curves in Figure 3 indicate.

One interpretation of the finding that predicted \( \mu_{23}^{\text{pl}} \) values for chemical PEG-protein interactions are in near-quantitative

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agreement with experimental results, not only for the smaller PEGs investigated but for larger PEGs as well, would be that neither chemical shielding \((\chi)\) effects nor excluded volume effects of large PEGs make significant contributions to the observed linear dependence of \(\mu_{23}\) 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 \(\mu_{23}\) 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...
Table II  Observed and Predicted Contributions of Chemical (Preferential) Interactions (PI) and Excluded Volume (EV) to PEG-Native Protein Interactions at 25 °C

<table>
<thead>
<tr>
<th></th>
<th>BSA b</th>
<th>Lysozyme c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interactions of small PEGs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical: contribution of a PEG interior group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed d</td>
<td>3.62 ± 0.36</td>
<td>0.47 ± 0.05</td>
</tr>
<tr>
<td>Predicted e</td>
<td>3.31 ± 0.23</td>
<td>0.80 ± 0.07 f</td>
</tr>
<tr>
<td>Chemical: contribution of 2 PEG end groups d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predicted b</td>
<td>2.07 ± 0.34</td>
<td>0.57 ± 0.09 f</td>
</tr>
<tr>
<td>Interactions of large PEGs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excluded volume, shielded chemical contributions per PEG residue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predicted excluded volume contribution i</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.75 ± 0.08</td>
<td>0.44 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Predicted reduction in chemical contribution from shielding i</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−0.86 ± 0.25</td>
<td>−0.21 ± 0.06 f</td>
<td></td>
</tr>
<tr>
<td>Comparison of observed and predicted μ23 per PEG residue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed k</td>
<td>2.94 ± 0.34</td>
<td>1.18 ± 0.24</td>
</tr>
<tr>
<td>Predicted l</td>
<td>3.2 ± 0.2</td>
<td>1.03 ± 0.07 f</td>
</tr>
</tbody>
</table>

Units of all entries are kcal mol⁻¹ (molal PEG residues)⁻¹.

a Uncertainty calculated as 1 standard deviation for linear least square fitting or propagated as described in Ref. 18.
b Na₁₄BSA c.
c Lysozyme⁻¹(Cl, phosphate)⁻¹.
d Slope dμ²₃/dN₃ of line fitted through the first 4 points (PEG 200–PEG 1000) of Figure 1 using predicted end-group contribution (see table) as the intercept.
e Slope dμ²₃/dN₃ of line fitted through the first 4 points (PEG 200–PEG 1000) of Figure 1 using predicted end-group contribution (see table) as the intercept.
f Predicted values calculated assuming the protein component is Lysozyme (see Supporting Information Tables SI and SII) 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.
g Low N₃ data are insufficiently accurate to obtain this from their intercept in Figure 1 (not shown).
h Intecept of red line in Figure 3, predicted from μ₂₃, β₂, and ASA (Figures 2A–2C).
i Predicted values calculated assuming the protein component is Lysozyme (see Supporting Information Tables SI and SII) 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.
j Predicted values calculated assuming the protein component is Lysozyme (see Supporting Information Tables SI and SII) 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.
k Slope dμ²₃/dN₃ of dashed line on Figure 1 (fitted through last 4 points, PEG 2000–PEG 6000).
l Slope dμ²₃/dN₃ of dashed line on Figure 1 (fitted through last 4 points, PEG 2000–PEG 6000).
m Predicted excluded volume contribution i 0.75

Predicting Excluded Volume Contributions to μ₂₃ for Interactions of Large PEGs with Proteins

Equations derived by Hermans²⁰ to predict the excluded volume between a flexible-coil polymer like high-N₃ PEG and a spherical protein or cylindrical DNA oligomer are summarized in Eqs. (3) and (4) (Table I). 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 = Δμ₂₃ (Ref. 19; see Table III below) The key prediction of this analysis is that excluded volume contributions to the μ₂₃ terms that contribute to PEG molal m-values are proportional to N₃ at high N₃ [Eqs. (3) and (4), Table I]. Using a statistical segment length (Kuhn length) Lₓ = 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-N₃ PEGs (Table II; see Supporting Information):
constant \( \chi_{N_3} \) to be determined by comparison of predicted and observed values of \( \mu_{23} \) for high-\( N_3 \) PEG. From the DNA 12-mer analysis (Supporting Information and Table III below), we find \( \chi_{N_3} = 0.6 \pm 0.06 \) (i.e. 40% of PEG residues are inaccessible to the 12-mer DNA oligomers). For lysozyme and BSA, a somewhat larger \( \chi_{N_3} = 0.74 \) provides a better fit to the experimental data, as summarized in Table II. Figure 3 plots \((1 - \chi_{N_3})\) \( \mu_{23} \), the reduction in \( \mu_{23} \) from this shielding effect, vs \( N_3 - 1 \) and shows that for both proteins this reduction in \( \mu_{23}^{PI} \) is comparable in magnitude to \( \mu_{23}^{EV} \). With \( \chi = 0.74 \) we obtain for high-\( N_3 \) PEGs:

\[
\begin{align*}
\text{FIGURE 3} & \quad \text{Dissection of unfavorable PEG-protein interactions into contributions from chemical} \\
& \quad \text{interactions, shielding, and excluded volume effects. Panel A: PEG-BSA interactions. Panel B: PEG-}
\quad \text{lysozyme interactions. Data points are the same as in Figure 1 plotted vs. } N_3-1. \text{ Red lines are pre-}
\quad \text{dicted contributions of unshielded preferential (chemical) interactions (PI) to } \mu_{23} \text{ from Eq. (2)} \\
& \quad \text{using } \alpha- \text{ and } \beta- \text{values from Figure 2}^{18} \text{ and ASA information from Supporting Information Table SI. Purple lines are predicted}
\quad \text{excluded volume (EV) contributions to } \mu_{23} \text{ of higher-}N_3 \text{ PEGs (PEG2000). Green lines are reduction due to shielding of chemical (preferential) interactions}
\quad \text{((1} - \chi)\text{PI) for a residue-accessibility factor } \chi = 0.74 \text{ (Table II). Blue lines are predicted}
\quad \text{combinations of shielded chemical and excluded volume contributions (} \chi \text{PI + EV) to } \mu_{23} \text{ for higher-}
\quad N_3 \text{ PEGs (PEG2000). Dashed black lines are sums of excluded volume (purple) and unshielded chemical}
\quad \text{(red) contributions (PI + EV).}
\end{align*}
\]

| Table III Contributions from Chemical (Preferential) Interactions (PI) and Excluded Volume (EV) to PEG Effects on DNA Duplex and Hairpin Melting |
|---------------------------------|----------------|----------------|
| **Observed PEG-DNA chemical interaction** | **12 bp Duplex\(^{ab}\)** | **4 bp Hairpin\(^{ab}\)** |
| Per PEG residue\(^c\) | \(-0.322 \pm 0.02\) | \(-0.160 \pm 0.02\) |
| Predicted excluded volume effect | Per PEG residue\(^d\) | \(0.36 \pm 0.03\) | \(0.061 \pm 0.006\) |
| Predicted reduction in PEG-DNA chemical interaction from shielding\(^e\) | | \(0.129 \pm 0.02\) | \(0.064 \pm 0.01\) |
| Observed, predicted PEG effects per PEG residue | Observed\(^f\) | \(0.168 \pm 0.01\) | \(-0.033 \pm 0.003\) |
| Predicted\(^g\) | \(0.164 \pm 0.037\) | \(-0.0346 \pm 0.013\) |

Units of all entries are kcal mol\(^{-1}\)(molal PEG residues\(^{-1}\)).

\(^a\) Data obtained at 40 °C.

\(^b\) Uncertainty calculated as 1 standard deviation for linear least square fitting or propagated as described in Ref. 18.

\(^c\) Slope \(d\mu_{23}/dN_3\) of line fitted through the first three points (EG-triEG) of Figure 4B and red lines of Figure 5.

\(^d\) Predicted from Eqs. (3) and (4) (Table I) using DNA and PEG structural information in Supporting Information Table SII.

\(^e\) Calculated as \(-1 - \chi_{N_3} \) \(\Delta\mu_{23}^{C} / N_3\) with \(\chi_{N_3} = 0.6\) (see text).

\(^f\) Slope \(d\mu_{23}/dN_3\) of dashed line on Figure 4C (fitted through last four points, PEG 2000–PEG20000, for duplex and last 5 points, PEG 1450–PEG20000, for hairpin).

\(^g\) \((\chi_{N_3} \Delta\mu_{23}^{C} + \Delta\mu_{23}^{EV}) / N_3\) predicted as in footnotes \(d\) and \(e\) with \(\chi_{N_3} = 0.6\). Corresponds to slope of blue line of Figure 5.
Lysozyme\(^\text{9,10}\) (Cl, phosphate)\(^\text{9}\) : \(\mu_{23} = \chi_{\infty} \mu_{23}^{\text{pi}} + \mu_{23}^{\text{ev}} = 1.03 \text{N}_3 - 0.17\)
\((\text{Na}^+)_{14}\text{BSA}\)^{14} : \(\mu_{23} = \chi_{\infty} \mu_{23}^{\text{pi}} + \mu_{23}^{\text{ev}} = 3.2 \text{N}_3 - 0.92\)

where the units of \(\mu_2\) are kcal mol\(^{-1}\) molal\(^{-1}\). The intercepts in Eq. (8) are negligible for high-N\(_3\) PEGs. If \(\chi_{\infty}\) is independent of N\(_3\) then Eq. (8) predicts that \(\mu_{23}\) is proportional to N\(_3\) for high-N\(_3\) PEGs. These predicted \(\mu_{23}\) 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 lysozyme. The observed result is \(\mu_{23} = 1.18 \text{N}_3\), which agrees with the prediction \((\mu_{23} = 1.03 \text{N}_3)\) within 10–15\% for this choice of \(\chi_{\infty}\).

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 \(\mu_{23} = 2.94 \text{N}_3\), in agreement with the prediction \((\mu_{23} = 3.2 \text{N}_3)\) within 5–10\% for this choice of \(\chi_{\infty}\).

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 \(\mu_{23}\).

**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 \(\mu_{23}\) between the melted strand(s) and the helix (m-value = \(\Delta\mu_{23}\), where from Eq. (5) \(\Delta\mu_{23} = \Delta \mu_{23}^{\text{pi}} + \Delta \mu_{23}^{\text{ev}}\)). This parallels the analysis of PEG-native protein interactions, quantified by values of \(\mu_{53}\) where \(\mu_{53} = \chi \mu_{23}^{\text{pi}} + \mu_{23}^{\text{ev}}\). 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 \(\chi_{\infty}\) is used for helix and melted strands).

The chemical term \(\Delta \mu_{23}^{\text{pi}}\) 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 melting.\(^\text{19}\) The excluded volume term \(\Delta \mu_{23}^{\text{ev}}\) 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, \(\mu_{23}\) 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 \(\mu_{23}\) values for each protein can be fit by a single line over the range of N\(_3\) investigated (Figure 1). Predictions of \(\mu_{23}^{\text{pi}}\) and \(\mu_{23}^{\text{ev}}\) in Table II and Figure 3 show that both are proportional to N\(_3\) at high N\(_3\), and that \(\mu_{23}^{\text{ev}}\) is a linear function of (nearly proportional to) N\(_3\) at low N\(_3\). Both \(\mu_{23}^{\text{pi}}\) and \(\mu_{23}^{\text{ev}}\) are positive (unfavorable).

Paralleling this behavior of PEG-protein interactions, panels B and C of Figure 4 show that PEG m-values = \(\Delta\mu_{23}\), 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 monomodal PEG m-values (i.e. \(\Delta\mu_{23}/\text{N}_3\)), which to a good approximation are independent of N\(_3\) at both small and large N\(_3\) (see Figure 4A)). Comparison of Figure 4B, C with Figure 1 shows a key difference between the low-N\(_3\) PEG-protein and PEG-DNA data. PEG-DNA \(\Delta\mu_{23}\) values are negative for EG, and become increasing negative with increasing N\(_3\) between EG and tetraEG by \(-0.32\) kcal mol\(^{-1}\) (molal PEG residues\(^{-1}\)) for duplex and \(-0.16\) kcal mol\(^{-1}\) (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 (Figure 2, Table II). The two-fold difference in PEG effects for 12-bp duplex vs. 12-mer hairpin melting is consistent with the ratio of \(\Delta\text{ASA}\) of melting for duplex and hairpin, and supports the interpretation of these effects as purely chemical interactions.

In the vicinity of tetraEG, PEG-DNA \(\Delta\mu_{23}\) 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 \(\Delta\mu_{23}\) 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 III. 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 (Δμ23) of high N3 PEGs on duplex and hairpin melting in Table III and Figures 5A and 5B (analogous to Figure 3) show that these quantities are positive (unfavorable; helix stabilizing) and proportional to N3. The predicted excluded volume effect is much larger for duplex melting (0.36 kcal mol\(^{-1}\) (molal PEG residues)\(^{-1}\)) than for hairpin melting (0.061 kcal mol\(^{-1}\) (molal PEG residues)\(^{-1}\)) primarily because of the stoichiometry of duplex melting, forming two strands from one duplex.

The behavior of Δμ23 for DNA melting as a function of PEG N3 at high PEG N3 predicted from Δμ\(^{\text{pl}}\)\(_{23}\) + Δμ\(^{\text{ev}}\)\(_{23}\) (dotted lines in Figures 4D and 4E) agrees qualitatively but not quantitatively with the observed Δμ23. We attributed the difference to shielding of a fraction (1 – χ) of PEG residues in the interior.

**FIGURE 4** Analysis of m-values quantifying effects of the monomer-polymer series of PEGs (EG to PEG20000) on stability of DNA helices.\(^{19}\) Panel A: plot of monomolal PEG m-values (i.e., m-value/N3 = Δμ23/N3) vs. logN3. Panel B: plot of molal scale PEG m-values (Δμ23) vs. number of interior PEG repeats (N3 – 1) for small and intermediate PEGs (N3 ≤ 14). Lines for N3 ≤ 3 are linear fits of EG, diEG and triEG m-values. Lines for N3 > 13 are from panel C. Panel C: plot of molal scale PEG m-values (Δμ23) vs. number of interior PEG repeats (N3 – 1) for all (N3 – 1) investigated. Lines are best fits to four (duplex) and five (hairpin) highest-N3 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 (Δμ23). Panel B: PEG-hairpin m-values (Δμ23). Red lines are predicted contributions of unshielded preferential (chemical) interactions (PI) to Δμ23 calculated using observed lower-N3 (EG-triEG) slopes from Figure 4B. Purple lines are predicted excluded volume (EV) contributions to Δμ23 of higher-N3 PEGs (≥PEG1450). Green lines are reduction due to shielding of chemical (preferential) interaction contributions ((1 – χ)PI) for a residue-accessibility factor χ = 0.6 (Table III). Blue lines are predicted combinations of shielded chemical and excluded volume contributions (χPI + EV) to Δμ23 for higher-N3 PEGs (≥PEG1450). Dashed black lines are sums of excluded volume (purple) and unshielded chemical (red) contributions (PI + EV).
of PEG flexible coils, reducing the per-residue interaction of PEG with DNA.\textsuperscript{19} Table III and Figures 5A and 5B show that quantitative agreement is obtained for $\gamma_\infty \approx 0.6$ (blue lines on Figure 5), similar to the best fit value for PEG-protein interactions ($\gamma_\infty \approx 0.74$).

**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.\textsuperscript{5,6,13,21–27}

The PEG-protein interactions summarized in Figure 1 were originally interpreted as a steric (excluded volume) effects.\textsuperscript{6} 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 proteins.\textsuperscript{13} 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 ($\Delta m_{23}$). 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 contributions.\textsuperscript{28–30} 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 $m$-values.\textsuperscript{31–33} Here we determine chemical interactions from small compound data,\textsuperscript{18} and calculate excluded volume interactions of large PEGs with proteins and nucleic acids using Hermans’ theory.\textsuperscript{30}

The analysis of effects of the entire PEG series on DNA helix formation (Figure 5; see also Knowles et al.\textsuperscript{19} 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 $\mu_{23}$ or $\Delta \mu_{23}$ (i.e. $m$-values) for all PEG sizes. Knowles et al.\textsuperscript{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.\textsuperscript{18} demonstrated that an interpretation based entirely on chemical interactions is sufficient to interpret $\mu_{23}$ 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 formation,\textsuperscript{34} and effects of urea, glycin betaine, proline and other small solutes on protein folding.\textsuperscript{35–38} Likewise, an interpretation based entirely on chemical interactions is sufficient to interpret $\mu_{23}$ values for the interactions of urea, proline and glycine betaine with a variety of small model compounds.\textsuperscript{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\textsuperscript{18,19} provides a general method to separate and interpret chemical ($\Pi$, $\gamma$) and excluded volume interactions of all PEGs with biopolymers, as well as chemical and excluded volume effects of all PEGs on any biopolymer process.

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**REFERENCES**


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