Quantitative Model of the Phase Behavior of Recombinant pH-Responsive Elastin-Like Polypeptides

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Quantitative models are required to engineer biomaterials with environmentally responsive properties. With this goal in mind, we developed a model that describes the pH-dependent phase behavior of a class of stimulus responsive elastin-like polypeptides (ELPs) that undergo reversible phase separation in response to their solution environment. Under isothermal conditions, charged ELPs can undergo phase separation when their charge is neutralized. Optimization of this behavior has been challenging because the pH at which they phase separate, pHc, depends on their composition, molecular weight, concentration, and temperature. To address this problem, we developed a quantitative model to describe the phase behavior of charged ELPs that uses the Henderson–Hasselbalch relationship to describe the effect of side-chain ionization on the phase-transition temperature of an ELP. The model was validated with pH-responsive ELPs that contained either acidic (Glu) or basic (His) residues. The phase separation of both ELPs fit this model across a range of pH. These results have important implications for applications of pH-responsive ELPs because they provide a quantitative model for the rational design of pH-responsive polypeptides whose transition can be triggered at a specified pH.

Introduction

New materials capable of undergoing bioresponsive self-assembly and higher-order supramolecular organization are required to build the next generation of biomaterials. Genetic engineering is a promising approach to synthesize such materials, and guided by this belief, we have been exploring recombinant elastin-like polypeptides (ELPs) as building blocks for self-assembled nanoparticles for drug delivery,1-3 hydrogels for local drug delivery,4 and tissue engineering.5-8 ELPs are recombinant protein—polymers composed of pentapeptide (Val-Pro-Gly-Xaa-Gly) repeat units and are so named because this pentamer and its analogs are recurring motifs in tropoelastin in a wide range of species.9 ELPs undergo an inverse phase transition, also called a lower critical solution temperature (LCST) transition, at a characteristic temperature, Tc, above which they phase separate from bulk water. At the molecular level, the identities of Xaa and L control this phase behavior.10 When pH-sensitive acidic or basic amino acids are placed at some of the Xaa positions, the Tc of these ELPs becomes dependent on pH.

Polymers that show LCST behavior are one class of materials that can be used as the building blocks for bioresponsive systems,11 and we have chosen to focus on ELPs for the following reasons: ELPs are genetically encodable, so they can be produced in heterologous expression systems with high yield and purity. The ability to produce ELPs recombinantly has several important ramifications. First, we have found that ELP fusion proteins also retain stimulus-responsive behavior. The ability to impart stimulus responsiveness to proteins and peptides by gene-level fusion of an ELP tail is a simple method to create proteins and peptides whose physical behavior (e.g., solubility) can be externally modulated by a small change in solution conditions. This attribute is extraordinarily useful because it generates a range of applications in biotechnology, ranging from nonchromatographic purification of ELP fusions12-14 to the development of affinity capture reagents15-17 and interfaces.18-20 ELPs are also attractive for biomedical applications21-24 including drug delivery11,23,25-29 and tissue engineering5-8 because they are biocompatible,30,31 nontoxic, and biodegradable.3,27 For biomedical applications in which ELPs are injected or implanted in vivo, genetically encoded synthesis provides significant advantages over synthetic polymers that display LCST behavior because it provides precise control over their composition, molecular weight (MW), and polydispersity, features that control their in vivo biodistribution, biodegradation, and disposition.25,32

In an effort to diversify the range of applications of ELPs, we have recently focused on the use of pH as a trigger of their phase-transition behavior.33 This effort was motivated by the recognition that pH plays a role in many biological processes and is hence a useful trigger to develop bioresponsive therapeutics. For example, the extravascular space within tumors has a reduced pH compared with that found in blood or many healthy tissues because of tumor hypoxia and production of lactate by anaerobic glycolysis. A large percentage of aggressive clinical tumors display regions with elevated levels of lactic acid,34 and areas of lactic acid production can be diffusely spread across large regions of the tumor. The elevation in lactic acid level correlates with the lower pH of tumors.35 There is, however, considerable variability between tumor types; sarcoma and adenocarcinoma have pH values as low as 5.6, squamous...
cell carcinoma have pHs as low as 6.3, and melanoma have been measured as low as pH 6.8.35

Because ELPs undergo a sharp phase change that can be isothermally triggered by a small change in pH, and because this pH responsiveness is controlled by the type and number of ionizable residues and MW of the ELP, in principle, it should be possible to synthesize ELPs that are designed to undergo their phase transition within a narrow, physiologically relevant range of pH that is optimized for delivery to a specific tumor type. This level of control of the pH responsiveness of ELPs or other stimulus-responsive polymers requires a biophysical model that is capable of predicting the pH responsiveness of these polymers with great precision. However, to the best of our knowledge, a quantitative model that allows prediction of the pH at which a charged ELP will undergo its phase transition does not exist. In an effort to address this limitation, we report herein a quantitative model that incorporates the effect of pH with MW and solution concentration, the two other primary variables that control the phase-transition behavior of an ELP, to predict quantitatively the $T_c$ of ionizable ELPs.

Materials and Methods

ELP Biosynthesis and Purification. ELPs were synthesized by heterologous expression of a plasmid-borne synthetic gene in E. coli, as previously described (Figure 1 of the Supporting Information). Genes encoding ELPs were constructed using recursive directional oligonucleotides from Integrated DNA Technologies (Corvalis, IA). All DNA plasmids were purified using Qiaprep spin miniprep kits (Novagen, Madison, WI). We purchased 5′-phosphorylated oligonucleotides linearized vector was generated by incubation with $Bgl$ II and $Pflm$ I, and the correct insert was purified by gel extraction. The vector and annealed oligonucleotide cassette were purified using a QIAquick PCR purification kit (Qiagen, Germantown, MD). The vector and annealed oligonucleotide cassette were ligated as previously described.25 In a typical round of RDL, linear inserts were generated by restriction digestion of plasmid DNA with Pflm I and Bgl II, and the correct insert was purified by gel extraction. The linearized vector was generated by incubation with Pflm I; the linearized vector was dephosphorylated using calf intestinal phosphatase (CIP) and then purified using a PCR purification kit. The linearized vector and insert were then ligated to obtain successively longer synthetic genes. After the desired number of rounds of RDL to oligomerize the monomer gene to the desired number of repeats, the insert gene was obtained by restriction digestion, as described above, and ligated into a modified pET25b+ expression vector that was linearized by digestion with $SfiL$. This approach produced synthetic genes (Table 1 of the Supporting Information) that express acidic ELPs with the sequence MSKGPG[XGVPG]$_L$=40,80,160 WPC with $X$ = V/I/E [1:3:1] and basic ELPs with the sequence MSKGPG[XGVPG]$_L$=40,60,100,120 WP with the ratio of $X$ = V/H/G/A [1:2:1:1]. Successful ligation products were transformed into chemically competent BLR(DE3) (Novagen, Madison, WI) cells for ELP expression.

To express ELPs, 1 L cultures of TB (MoBio; Carlsbad, CA) were seeded from 50 mL of overnight cultures (100 µg/mL ampicillin) and incubated for 24 h at 37 °C and ∼210 rpm. Bacterial cultures were centrifuged, resuspended in ∼20 mL of PBS, and disrupted by probe ultrasonication (Misonix, Farmingdale, NY). To precipitate DNA with the insoluble debris, the lysate was supplemented with polyethyleneimine to a concentration of ∼1% and centrifuged at 16 100 RCF at 4 °C. The clarified supernatant was removed, supplemented with up to 3 M NaCl, as required to induce the ELP transition, and purified by inverse transition cycling (ITC).912 In brief, ITC consists of raising the NaCl concentration as needed (0 to 3 M) to induce ELP phase separation. The ELP pellet was collected by hot centrifugation at 16 100 RCF (37 °C). The enriched pellet was resuspended in buffer and centrifuged in the cold (4 °C) to remove aggregated contaminants. The enriched ELP solution was subjected to this cycle of hot and cold centrifugation four to six times until sufficient purity was obtained. ELP purity was confirmed using SDS-PAGE (Figure 2 of the Supporting Information). The ELP concentration was determined using UV−vis spectrophotometry and an estimated molar extinction coefficient for the sole tryptophan26 in each ELP of 5690 cm$^{-1}$ M$^{-1}$ at 280 nm. Yields of purified ELP were ∼100 mg/L culture.

To determine the pH-dependent transition temperature, concentrated ELPs were dialyzed into buffer solutions containing pH-adjusted solutions of sodium succinate (pH 6.4) or sodium phosphate (pH ≥ 6.4). Both buffers were selected on the basis of the relative insensitivity of their $pK_a$ to temperature. For the acidic ELPs, buffers were prepared with 10 mM buffer and 140 mM NaCl. The basic ELPs were prepared with 100 mM buffer and 50 mM NaCl because they have a higher linear charge density than the acidic ELPs and hence required additional buffering capacity to maintain their pH. We determined ELP transition temperatures on a CARY 300Bio UV−vis spectrophotometer (Varian, Palo Alto, CA) by scanning the temperature at 1 °C/min. The transition temperature was defined as the solution temperature that corresponded to the maximum first derivative of the optical density at 350 nm (Figure 3 of the Supporting Information).

Results

pH Dependence of the ELP Phase Transition. We synthesized two ELP libraries with a range of MWs that have pH-sensitive phase behavior. The first library comprises acidic ELPs that contain multiple glutamic acid residues that repeat along the ELP sequence. At low pH, these basic ELPs are charged and have a high phase-transition temperature, but a decrease in pH below their $pK_a$ leads their glutamic acid residues to be protonated and become neutral, which dramatically reduces their phase-transition temperature. The second library contains the basic amino acid histidine interspersed periodically along the ELP sequence. Above their $pK_a$, they are soluble; however, when the pH rises above their $pK_a$, they become neutralized, and their phase-transition temperatures decrease. Therefore, these two ELP libraries exhibit opposing pH-dependent phase-transition behavior across a range of pH (Figure 1). These pH-triggered ELPS exhibit a phase transition over a wide range of pH that was deliberately chosen to test the robustness of a quantitative model.

As has been previously reported, the phase-transition temperature for nonionic ELPs strongly depends on both the ELP
chain length and its solution concentration,\textsuperscript{32} so that these orthogonal variables allow the phase-transition temperature to be finely tuned. We observed that this relationship also holds upon introducing pH-sensitive guest residues to an ELP but that the effect of the pH on the phase-transition temperature dominates over the ELP chain length or its solution concentration. To describe this finding within a quantitative framework, we developed an empirical analytical model that fit the observed pH dependence of the transition temperature (\(T_{t}\), in degrees Celsius) of the ELP phase transition of both ELP libraries as a function of their concentration (\(C_{\text{total}}\), in micrometers) and length (\(L\), in pentamers). This model explicitly accommodates the pH-dependent behavior of both the acidic and basic ELP libraries. A key assumption of this model is that the transition temperature at an intermediate pH can be linearly interpolated between the transition temperature of a fully deprotonated ELP at a high pH, \(T_{\text{depro}}\), and a fully protonated ELP at a low pH, \(T_{\text{pro}}\), as follows

\[
T_{t} = f_{\text{depro}}T_{\text{depro}} + (1 - f_{\text{depro}})T_{\text{pro}}
\]  

where \(f_{\text{depro}}\) is the fraction of total ELP guest residues that is deprotonated. This linear approximation is strongly supported by previous observations, which showed that the transition temperature depends linearly on the mixture of both charged and neutral guest residues.\textsuperscript{9}

To determine \(f_{\text{depro}}\), the Henderson–Hasselbalch equation can be used to estimate the relative concentration of protonated, \(C_{\text{pro}}\), versus deprotonated, \(C_{\text{depro}}\,\text{ guest residues in a polymer solution as follows}

\[
\text{pH} = pK_{a} + \log \left( \frac{C_{\text{depro}}}{C_{\text{pro}}} \right)
\]

By conservation of total ionizable residues, \(C_{\text{total}}\)

\[
C_{\text{total}} = C_{\text{depro}} + C_{\text{pro}}
\]

Substituting eq 3 into eq 2 and rearranging provides an expression for the fraction of guest residues protonated in the ELP polymer solution.

\[
f_{\text{depro}} = \frac{C_{\text{depro}}}{C_{\text{total}}} = \frac{1}{1 + 10^{(pK_{a} - \text{pH})}}
\]

By substituting eq 4 into eq 1 and rearranging, the following approximation is obtained

\[
T_{t} = T_{\text{pro}} + \frac{T_{\text{depro}} - T_{\text{pro}}}{1 + 10^{(pK_{a} - \text{pH})}}
\]

The above equation linearly interpolates between the transition temperature of a fully protonated and a fully deprotonated ELP as a function of pH and was explored in combination with other variables that influence the transition temperature. Note that eq 5 is valid for fitting the behavior of ELPs with either acidic or basic guest residues. Basic amino acids such as histidine are charged in their protonated form; therefore, their transition temperatures decrease above their p\(K_{a}\). In contrast, acidic amino acids such as glutamic acid are neutral in their protonated state; therefore, their transition temperatures increase above their p\(K_{a}\). Therefore, eq 5 describes the pH-dependent phase-transition behavior of both basic and acidic ELPs.

\(T_{t}\) Dependence on Concentration at Fixed Length. Prior to developing a single multivariate model that relates transition temperature to pH, length, and concentration, we first verified that eq 5 is valid at fixed length; furthermore, this step was necessary to confirm our assumption that the p\(K_{a}\) is roughly independent of the ELP length. Empirical observation has shown that the ELP transition temperature depends on the natural logarithm of the polymer concentration; furthermore, the degree of this dependence is strongly influenced by the selection of guest residues and polymer length.\textsuperscript{32} The following relationship has been previously derived to quantify the concentration dependence for ELPs\textsuperscript{32}

\[
T_{t} = T_{\text{ref}} - b \ln[C]
\]

where \(T_{\text{ref}}\) is the transition temperature at a reference concentration (1 \(\mu\text{M}\) here) and ELP concentration, \(C\), is in units of mM. The slope, \(b\), represents the concentration dependence of the transition temperature. When eq 6 is written for the protonated (\(T_{\text{pro}}^{\text{ref}}, b_{\text{pro}}\)) and deprotonated (\(T_{\text{depro}}^{\text{ref}}, b_{\text{depro}}\)) forms of the ELP and inserted into eq 5, the following relationship is obtained

\[
T_{t} = T_{\text{pro}}^{\text{ref}} - b_{\text{pro}} \ln[C] + \frac{T_{\text{depro}}^{\text{ref}} - T_{\text{pro}}^{\text{ref}} - (b_{\text{depro}} - b_{\text{pro}}) \ln[C]}{1 + 10^{(pK_{a} - \text{pH})}}
\]

The above equation was used to fit each ELP analyzed in our data set (Figure 1) to obtain the best-fit parameters (Table 1). Interestingly, these data suggest that all parameters of eq...
constant within its compositional library. This leads us to propose the assumption that the pKs of acidic and basic ELPs follow this principle, we fit both libraries to eq 8 at a number of fixed pH values (Table 2). The fit parameters demonstrate that Tk and k both depend on pH; however, Cc does not substantially change with pH. This is partially due to the fact that Cc is an extrapolated value, far above the concentration that is achievable in a dilute, buffered solution. Despite this, it appears that at constant pH, eq 8 accurately describes the transition behavior of both acidic and basic ELPs (Figure 2).

TKi, dependence on concentration, length, and pH. Having demonstrated that the ELP phase behavior is simultaneously pH-, length-, and concentration-dependent, it is now possible to develop a simple equation that accounts for each of these behaviors. To do this, a form of eq 8 that represents both the protonated and deprotonated ELP can be substituted into eq 5 and rearranged, yielding

\[
T_i = T_{c,\text{pro}} + \frac{k_{\text{pro}}}{L} \ln \left( \frac{C_{c,\text{pro}}}{C} \right) + \frac{T_{c,\text{depro}} - T_{c,\text{pro}}}{L} \left( \frac{k_{\text{depro}}}{L} \ln \left( \frac{C_{c,\text{depro}}}{C} \right) - \frac{k_{\text{pro}}}{L} \ln \left( \frac{C_{c,\text{pro}}}{C} \right) \right) \left( 1 + 10^{(pK_a - pH)} \right)
\]

where \( C_{c,\text{depro}} \) and \( C_{c,\text{pro}} \) are the critical concentrations for the deprotonated and protonated polymers, respectively, \( T_{c,\text{depro}} \) and \( T_{c,\text{pro}} \) are the critical transition temperatures for the deprotonated and protonated polymers, respectively, and \( k_{\text{depro}} \) and \( k_{\text{pro}} \) are the parameters for the length-concentration interaction for the deprotonated and protonated polymers, respectively. Equation 9 assumes that pKs is independent of concentration and length, as supported by Table 1. Including pKs, eq 9 has seven parameters, so some simplification of this equation was considered to be desirable. The critical concentration, \( C_c \), is an extrapolated value, and it does not appear to have a significant dependence on pH (Table 2). Therefore, we simplified eq 9 by assuming that the critical concentrations are roughly equal between the protonated and deprotonated forms. After substitution of \( C_{c,\text{pro}} \) and \( C_{c,\text{depro}} \) with \( C_c \) into eq 9, we obtain

\[
T_i = T_{c,\text{pro}} + \frac{k_{\text{pro}}}{L} \ln \left( \frac{C_c}{C} \right) + \frac{T_{c,\text{depro}} - T_{c,\text{pro}}}{L} \left( \frac{k_{\text{depro}} - k_{\text{pro}}}{L} \ln \left( \frac{C_c}{C} \right) \right) \left( 1 + 10^{(pK_a - pH)} \right)
\]

The above relationship was fit to both the acidic and basic ELP libraries, spanning a range of concentration, \( C_c \), polymer length, L, and pH to determine the six parameters (Table 3). These parameters have been used to fit data as a function of

<table>
<thead>
<tr>
<th>ELP guest residues</th>
<th>length, L (pentamers)</th>
<th>pKs</th>
<th>( T_{c,\text{pro}}^{\text{eq}} ) (°C)</th>
<th>( T_{c,\text{depro}}^{\text{eq}} ) (°C)</th>
<th>( k_{\text{pro}} ) (°C/Ln [µM])</th>
<th>( k_{\text{depro}} ) (°C/Ln [µM])</th>
<th>R²</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>V/I/E [1:3:1]⁵</td>
<td>40</td>
<td>5.51(0.05)</td>
<td>36.9 (1.8)</td>
<td>148 (11)</td>
<td>2.48 (0.53)</td>
<td>15.5 (2.4)</td>
<td>0.990</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>5.51(0.05)</td>
<td>21.6 (2.3)</td>
<td>81.0 (2.0)</td>
<td>0.42 (0.67)</td>
<td>5.04 (0.54)</td>
<td>0.985</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>5.29 (0.03)</td>
<td>18.6 (1.1)</td>
<td>73.9 (0.8)</td>
<td>0.28 (0.32)</td>
<td>5.09 (0.23)</td>
<td>0.997</td>
<td>30</td>
</tr>
<tr>
<td>V/H/G/A [1:2:1:1]⁶</td>
<td>40</td>
<td>5.69 (0.09)</td>
<td>137 (12)</td>
<td>103 (4)</td>
<td>8.4 (2.0)</td>
<td>11.8 (0.9)</td>
<td>0.979</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>6.26 (0.06)</td>
<td>116 (6.7)</td>
<td>62.1 (2.8)</td>
<td>4.3 (1.1)</td>
<td>6.3 (0.6)</td>
<td>0.978</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>6.25 (0.05)</td>
<td>98.0 (4.6)</td>
<td>38.5 (2.9)</td>
<td>2.9 (1.0)</td>
<td>3.1 (0.7)</td>
<td>0.982</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>6.22 (0.05)</td>
<td>95.1 (4.4)</td>
<td>33.4 (2.8)</td>
<td>2.6 (1.0)</td>
<td>2.8 (0.7)</td>
<td>0.985</td>
<td>30</td>
</tr>
</tbody>
</table>

⁵ Data fit to eq 7 and parameters reported as the estimate (standard error). ⁶ Acidic ELP at pH 2.5~7.8, and concentrations from 5 to 100 µM. ⁷ Basic ELP at pH 5.5~8.0, and concentrations from 10 to 500 µM.
pH (Figure 3). Impressively, the global model fit describes \( \sim 97\% \) of the observed variability in the transition temperature for both libraries.

**Development of an Isothermal ELP Phase Diagram.** Some of the most interesting applications for pH-responsive ELPs are to develop smart biomaterials that respond to physiologically relevant pH gradients. Therefore, we note that a rearrangement of eq 10 can be used to estimate the isothermal transition pH at which these ELPs undergo their phase transition as a function of ELP concentration and chain length. The following rearrangement of eq 10 is solved for the inverse phase-transition pH, \( pHi \):

\[
pHi = pKa + \log \left( \frac{T - T_{c_{\text{depro}}} - \frac{k_{\text{depro}}}{L} \ln \left( \frac{C_c}{C} \right)}{T_{c_{\text{pro}}} - T + \frac{k_{\text{pro}}}{L} \ln \left( \frac{C_c}{C} \right)} \right)
\]

(11)

ELP chain length and concentration are simple to control; furthermore, the data presented in Table 3 can be used to select optimal conditions to optimize \( pHi \) within several pH units of the observed \( pK_a \) (acidic: \( pK_a = 5.36 \); basic \( pK_a = 6.28 \)). By plugging in the best-fit parameters obtained in Table 3, eq 11 can be used to estimate the pH at which a given ELP may transition at body temperature (Figure 4).

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**Table 2.** Dependence of the Transition Temperature on Length and Concentration at Fixed pH

<table>
<thead>
<tr>
<th>ELP guest residues</th>
<th>pH</th>
<th>( T_c (\degree C) )</th>
<th>( K (\degree C \text{ pentamers}) )</th>
<th>( C_c (\text{mM}) )</th>
<th>( R^2 )</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>V/I/E [1:3:1] ( ^b )</td>
<td>6.5</td>
<td>39.5 (2.7)</td>
<td>363 (72)</td>
<td>3.1 (2.7)</td>
<td>0.786</td>
<td>22</td>
</tr>
<tr>
<td>V/I/E [1:3:1] ( ^b )</td>
<td>5.5</td>
<td>32.8 (2.5)</td>
<td>278 (80)</td>
<td>1.8 (2.2)</td>
<td>0.841</td>
<td>14</td>
</tr>
<tr>
<td>V/I/E [1:3:1] ( ^b )</td>
<td>4.6</td>
<td>19.7 (1.0)</td>
<td>126 (27)</td>
<td>5.4 (6.7)</td>
<td>0.927</td>
<td>15</td>
</tr>
<tr>
<td>V/I/E [1:3:1] ( ^b )</td>
<td>3.7</td>
<td>13.3 (0.9)</td>
<td>106 (23)</td>
<td>7.8 (10.5)</td>
<td>0.929</td>
<td>15</td>
</tr>
<tr>
<td>V/H/G/A [1:2:1:1] ( ^c )</td>
<td>8.0</td>
<td>3.6 (1.2)</td>
<td>394 (24)</td>
<td>10.9 (3.4)</td>
<td>0.978</td>
<td>21</td>
</tr>
<tr>
<td>V/H/G/A [1:2:1:1] ( ^c )</td>
<td>7.4</td>
<td>8.5 (1.5)</td>
<td>403 (29)</td>
<td>9.9 (3.6)</td>
<td>0.968</td>
<td>21</td>
</tr>
<tr>
<td>V/H/G/A [1:2:1:1] ( ^c )</td>
<td>6.8</td>
<td>20.5 (1.2)</td>
<td>354 (26)</td>
<td>16.5 (6.0)</td>
<td>0.972</td>
<td>20</td>
</tr>
<tr>
<td>V/H/G/A [1:2:1:1] ( ^c )</td>
<td>6.4</td>
<td>30.9 (1.4)</td>
<td>348 (29)</td>
<td>20.9 (8.3)</td>
<td>0.964</td>
<td>19</td>
</tr>
<tr>
<td>V/H/G/A [1:2:1:1] ( ^c )</td>
<td>6.0</td>
<td>39.9 (1.5)</td>
<td>306 (31)</td>
<td>42.0 (22.7)</td>
<td>0.958</td>
<td>19</td>
</tr>
<tr>
<td>V/H/G/A [1:2:1:1] ( ^c )</td>
<td>5.5</td>
<td>58.7 (1.4)</td>
<td>340 (30)</td>
<td>24.8 (9.8)</td>
<td>0.958</td>
<td>13</td>
</tr>
</tbody>
</table>

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*Data fit to eq 8 and parameters reported as the estimate (standard error). \( ^b \) Acidic ELP lengths from 40 to 160 pentamers and concentrations from 5 to 100 \( \mu M \). \( ^c \) Basic ELP lengths from 40 to 120 pentamers and concentrations from 10 to 500 \( \mu M \).
We successfully developed two novel libraries of ELPs that phase separate near physiological temperature and pH; furthermore, one library (histidine) is soluble only at acidic pH, and the other (glutamic acid) is soluble only at basic pH. These amino acids were selected because they have titratable chemistries and pK_a values within the physiologically relevant range. When the amino acids become either cationic or anionic, the phase-transition temperature and solubility are increased. Histidine becomes protonated under acidic conditions, producing a cationic ELP with high solubility. In contrast, glutamic acid becomes deprotonated under basic conditions, conferring an anionic charge and enhancing ELP solubility. Lysine, aspartic acid, and tyrosine also have titratable chemistries, which may be useful to target phenomena that occur at pH values near their pK_a values. Presumably, lysine-containing ELPs would behave qualitatively similarly to histidine-containing ELPs, albeit at a much higher pH. In contrast, aspartic acid and tyrosine guest residues are expected to behave qualitatively similarly to the glutamic-acid-containing ELPs. Glutamic acid and histidine were chosen as the ionizable residues of interest because their pK_a values (4.3 and 6.1, respectively) are closest to the physiologically relevant range. The high pK_a values of tyrosine and lysine (10 and 10.5, respectively) place those responses outside of the relevant range, and while the pK_a of aspartic acid is similar to that of glutamic acid, the uncharged aspartic acid is less hydrophobic, meaning that protonation of this residue under acidic conditions would have less of an effect on the ELP T_c. Even though they have opposite responses to pH, our proposed model perfectly describes the behavior of both acidic and basic ELPs. Variations on this model may be applicable to other biopolymer phase transitions that depend on concentration or MW. Many other polypeptide assembly mechanisms depend on ionizable amino acids. For example, leucine zipper assemblies are pH-dependent because they are partially stabilized by electrostatic interactions between titratable amino acid side chains. These models may enable precise engineering of recombinant biopolymers that assemble at target physiological pH and temperature.

Conclusions

Herein we have described two libraries of ELPs with basic or acidic guest residues that have pH-responsive phase behavior. We observed that their concentration and MW influence their phase-transition behavior and then developed an empirical model that describes the ELP phase-transition temperature as a function of concentration, chain length, and pH. This approach is valid for ELPs that undergo phase separation at high pH (histidine-containing ELPs) and low pH (glutamic acid-containing ELPs). These results are useful because they will enable the rational design of ELPs that are capable of exhibiting phase-transition behavior in response to a specified change in pH as the trigger. Future work will examine the generality of this model with respect to ELPs that contain other ionizable residues not used to develop the model (e.g., Asp, Lys) as well as the validity of this model in predicting the pH-triggered self-assembly of diblock ELPs that contain one ionizable block, with the goal of rational design of ELPs that exhibit self-assembly into nanoscale structures such as micelles or vesicles or conversely exhibit pH-triggered disassembly in response to pH gradients that exist in physiological systems.

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Supporting Information Available. Additional characterization of the polymers analyzed within this manuscript. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes