Nonlinear Mechanical Force Induced pK_a Shifts: Implications for Efficiency of Conversion to Chemical Energy

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The nonlinear conversion of mechanical energy into chemical energy is demonstrated by one member of a series of elastic protein-based polymers that exhibit inverse temperature transitions of hydrophobic folding and assembly as the temperature is raised from below to above the transition temperature range. When these protein-based polymers contain amino acid residues with ionizable side chains, such as the carboxyl of a glutamic acid residue (Glu, E), and are γ-irradiation cross-linked to form elastic matrices, the matrix is swollen, i.e., the polymers are unfolded and disassembled when the COO^- moiety is present. On lowering of the pH to form the COOH moiety, the matrix isothermally contracts due to hydrophobic folding and assembly and, on doing so, the matrix can perform the mechanical work of lifting a weight.[1,2] Significantly, polymers of the composition poly[f_V(GVGIP),f_E(GEGIP)], where f_V and f_E are mole fractions with $f_V + f_E = 1$ and G, V, and P are the glycine (Gly), valine (Val), and proline (Pro) residues, respectively, exhibit hydrophobic-induced increases in pK_a as f_E approaches 0.[3] As reported here, on application of a force, f, to stretch the cross-linked, contracted matrix with an f_E of 0.18, a dramatic nonlinear increase in the pK_a of the carboxyl is observed with increasing applied force. As the ΔpK_a can be equivalent to the change in chemical energy, ΔE, of picking up protons, this means for the condition of constant temperature, T, and pressure, P, that the $(\partial E/\partial f)_{T,P}$ increases with increases in force, that is, the efficiency of conversion of mechanical energy into chemical energy increases remarkably at higher forces.

The synthesis of poly[0.82(GVGIP),0.18(GEGIP)] has been described previously;[4] the procedure for 20 Mrad γ-irradiation cross-linking this polymer to form the elastic matrix also has been described previously,[5] and the apparatus in which the acid/base titrations were carried out with the matrix extended at a fixed force is as previously used.[6] The titrations were carried out at 23 °C using 90 min/data point, requiring 30−35 h to complete a single titration. The experiment is to extend the elastomer at low pH and at constant temperature to a given force and to carry out the acid/base titration, Δn_i, while increasing the length, l_i, in order to maintain the force constant, f_i, that is, $(\partial l_i/\partial n_i)_{f_i,T}$. Characteristically, the length increased by approximately a factor of 2 during the titration. The several constant loads were 0, 1.0, 1.5, 1.75, 2.0, and 2.2 g, which for the particular elastomeric band were 0, 3.6, 5.4, 6.4, 7.3, and 8.0 × 10^5 dynes/cm^2, respectively. The data reported involves

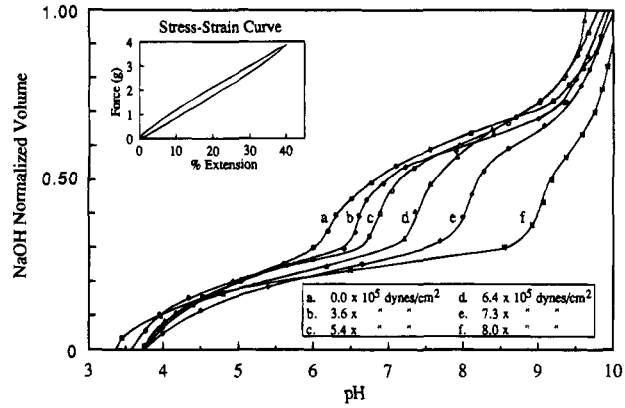

Figure 1. Acid/base titration curves for an elastomeric band of γ-irradiation cross-linked poly[0.82(GVGIP),0.18(GEGIP)] at different extending forces: 0, 3.6, 5.4, 6.4, 7.3, and 8.0 × 10^5 dynes/cm^2. The effect of increasing mechanical force is a dramatic increase in the pK_a of the glutamic acid (Glu, E) carboxylic side chain. Inset: The stress/strain data for the same elastomeric band showing a near linear curve. In this inset, a 2.2 g force is equivalent to 8 × 10^5 dynes/cm^2. See text for discussion.

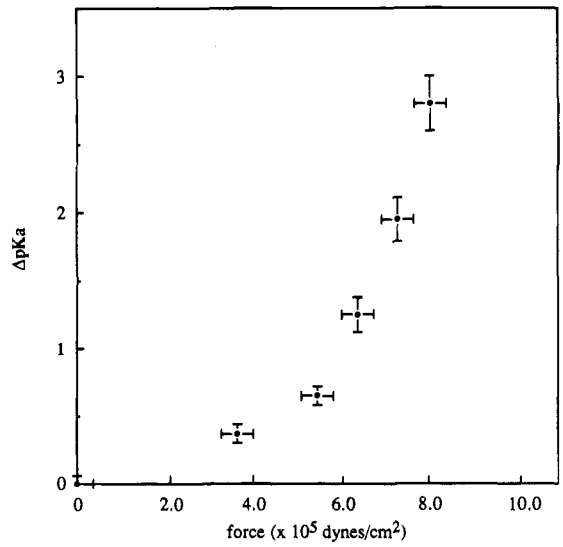

Figure 2. Plot of the data of Figure 1 in terms of the ΔpK_a with a zero force pK_a value of 6.2 as the zero reference value. A remarkably nonlinear ΔpK_a versus mechanical extending force is observed. See text for discussion.

18 titrations on a single elastomeric strip. Similar data was demonstrated on a second strip of the same composition.

Representative titration curves are shown in Figure 1, and a plot of ΔpK_a versus applied force is given in Figure 2, where each data point is given as the mean and standard deviation of three titrations. The titrations were reversible; that is, the value of the pK_a obtained for the titration at a 0 or 1 g load was unchanged after a titration was carried out at a 2.2 g load. Clearly, as the force is increased, a very nonlinear increase in pK_a is observed. Also included as an inset in Figure 1 is the essentially linear stress/strain curve over the force range of interest for the cross-linked, contracted state of the poly[0.82-(GVGIP),0.18(GEGIP)] matrix at low pH. The force increases by approximately 1 g for each 10% of extension.

The chemical potential, μ, is defined as $\mu = RT \ln a$ where a is the activity. At the low proton concentrations used here $[H^+] = a$, such that $\ln a = \ln[H^+] = 2.3 \log[H^+] = -2.3$ pH. Accordingly, the chemical potential becomes $-2.3RT$ pH. The change in proton chemical potential, $\Delta\mu_i$, required to maintain 50% ionization, i.e., to maintain the system at its pK_a with the

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degree of ionization, $\alpha = 0.5$, becomes $-2.3RT\ \Delta pK_a$. From Figure 1, the change in proton chemical potential due to changes in force at constant α and T is seen to be a negative quantity, i.e., $(\partial\mu_i/\partial f_i)_{\alpha,T} < 0$. On the other hand, the charge−charge repulsion mechanism for inducing pK_a shifts results in the opposite sign for the partial derivative,[7] i.e., $(\partial\mu_i/\partial f_i)_{\alpha,T} > 0$. Therefore, the pK_a shifts reported here are not due to the usually considered electrostatic mechanism.[7−13]

The mechanism for the stretch-induced pK_a shifts in these polymers that exhibit inverse temperature transitions of hydrophobic folding and assembly on raising the temperature has been termed an apolar−polar repulsive free energy of hydration.[1,6] On extension, the only change in composition is an increase in the amount of water in the matrix. In spite of this increase in the water content of the matrix, formation of the more polar COO^- is energetically less favored. Accordingly, the reason for the increase in pK_a must be that the water entering the matrix is not suitable for hydration of the charged COO^- moiety. As the polymers are hydrophobically folded and assembled within the contracted matrix, extension causes the hydrophobic side chains to become exposed and surrounded by waters of hydrophobic hydration. The conclusion, therefore, is that waters of hydrophobic hydration are not suitable for hydration of charged species. Thus, there is a competition for hydration between the apolar (hydrophobic) and polar groups causing a delay in the formation of COO^- until the pH is further increased, that is, until a lower proton activity is reached.

This competition for hydration resulting in a ΔpK_a has been observed in poly[f_V(GVGIP),f_X(GXGIP)] for X = E (Glu),[3] D (Asp),[14] and K (Lys)[15] with f_X varied from 1.0 to 0.06 where, in a nonlinear way, the ΔpK_a becomes greater as f_X approaches 0, and it has been observed in polytricosapeptides with five more-hydrophobic Phe residues replacing less-hydrophobic Val residues.[16,17] Furthermore, as the number of Val residues replaced by Phe residues increases from 2 to 3 to 4 and to 5, there is also observed a very nonlinear pK_a shift with the number of Phe residues.[18] This nonlinear hydrophobic induced pK_a shift was interpreted to mean that the more efficient conversion of an energy input that caused a change in hydrophobicity to the chemical energy output of picking up or releasing protons, that is, of "pumping protons", occurs when the system is hydrophobically "poised".[19]

Indeed, as demonstrated in Figure 2, when the input is mechanical energy, the chemical energy output ($\Delta E = (\Delta\mu_i)\cdot(\Delta n_i) = -2.3RT(\Delta pK_a)(\Delta n_i)$) is small when the load changes from 0 to 1 g, but the chemical energy output is much larger when the same mechanical energy input occurs on going from a load of 1 to 2 g. Thus, poising is also relevant, when using these polymers that exhibit inverse temperature transitions, to mechano-chemical transduction; that is, for the most efficient conversion of mechanical energy to chemical energy by this mechanism, the change in mechanical force should occur at higher force levels; that is, $(\partial E/\partial f)_{T,P}$ is larger at higher forces.

Free energy transductions using protein-based polymers capable of inverse temperature transitions have previously been demonstrated involving each of the intensive variables of the free energy of mechanical force, pressure, temperature, chemical potential, electrochemical potential, and electromagnetic radiation.[1,20] Changes in each of the latter five intensive variables have resulted in the performance of mechanical work, and the conversion of electrochemical energy into chemical energy and of light into chemical energy have also been demonstrated[21,22] with correctly designed protein-based polymers. What are being developed here are the principles for protein-based polymer engineering required in order to design polymers for the most efficient energy conversion. The relevance of these results to mechanochemical transduction in biology, e.g., stretch-activated channels[23] and receptors,[24] should be apparent.

In conclusion, molecular machines, composed of polymers that exhibit inverse temperature transitions of hydrophobic folding and assembly and capable of converting mechanical energy into chemical energy, achieve higher efficiencies for energy conversion when operating at higher force levels.

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