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Communications to the Editor

Effects of Pressure on the Behavior of the Thermoresponsive Polymer Poly(*N*-vinylisobutyramide) (PNVIBA)

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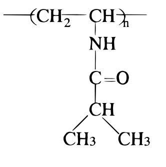
There are several known synthetic polymers, including vinyl polymers, that show a more or less distinct change in their molecular level aggregational states (in solution) and volume phase transitions (in gel form). Poly(*N*-isopropylacrylamide) (PNIPAM) and other polyacrylamide derivatives with both hydrogen-bonding properties and hydrophobic interactions in aqueous solutions are some of the best known examples.[1] These polymers show a sharp transition in their aggregation (collapse) state upon a change in temperature, and this change is reversible. The swelling and shrinking of gels prepared from such polymers have been extensively studied, both experimentally and theoretically.[2] Several applications such as drug delivery and bioseparation have been studied by taking advantage of these properties.[3]

Recently, Akashi *et al.* have synthesized a series of new polymers based on poly(vinylamine), which have dramatic thermoresponsive properties.[4,5] Basically, these polymers have amide bonds in their side chains in an inverted direction to the corresponding acrylamide derivatives. Poly(*N*-vinylisobutyramide) (PNVIBA:I), one of the homopolymers in this series, has shown a much sharper thermal transition in aqueous solution and a much larger volume change in gel form than PNIPAM (II).



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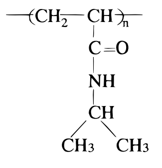

II

Besides the temperature, these thermoresponsive polymers can undergo transitions induced by other factors, such as salt concentration,[6] surfactant addition,[7] saccharide addition,[8] solvent,[9] etc. These changes recall the behavior of biological polymers such as proteins. Proteins undergo structural transformation induced by thermal changes, denaturant addition or, in some cases, salt concentration change and pH. PNIPAM has been considered to be a simple but relevant protein model and its thermodynamic properties have been investigated from such a point of view.[10,11]

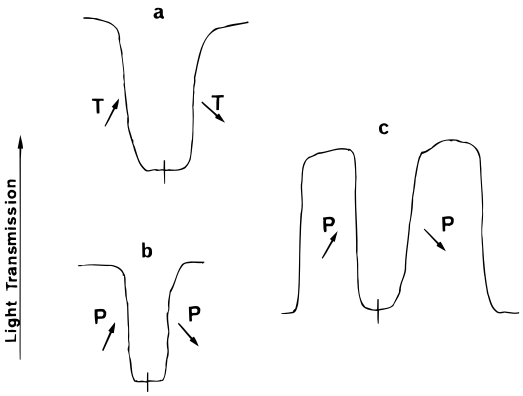

Figure 1. Light transmission change in PNVIBA solution (0.1 w/v %): (a) during temperature scanning at 100 MPa; (b) pressure scanning at 10 °C; (c) during pressure increases and decreases at 42 °C.

Proteins also change their structure following exposure to extreme high pressure, with the critical value of the pressure being dependent on the nature of the protein.[12] We have previously investigated pressure-induced changes in protein structure and function in several examples.[13] PNIPAM derivatives have been shown to undergo pressure-induced transitions, both in solution[14] and in gel form.[15]

In this communication, we measured the pressure dependence of the thermal transition of PNVIBA aqueous solution, plotted the relevant temperature−pressure diagrams, and discussed the thermodynamic implications of these results.

Materials and Methods. PNVIBA was synthesized as previously reported.[5] NIPAM monomer was kindly donated by Kojin Co. (Tokyo, Japan), which was polymerized by a radical initiator after purification through precipitation from alcohol. Their molecular weights and molecular distributions were determined chromatographically (Shodex AD-80M/S column in DMF solution, standardized against PEG−PEO); $M_n = 28$K and $M_w/M_n = 2.8$ for PNVIBA, and $M_n = 14$K and $M_w/M_n = 3.3$ for PNIPAM. These molecular weights are above the minimum domain size for PNIPAM to show an all-or-none transition.[11]

The cloud points for the aqueous solutions of these polymers were determined by observing light transmission. A high-pressure optical cell was constructed by Teramecs Co. (Kyoto, Japan). Experiments were done by either scanning the temperature by an electronic regulator at constant pressure or scanning the pressure by a compact hand-driven high-pressure pump at constant temperature. There was practically no concentration dependence of the cloud temperature/pressure below ca. 1% (w/v).

Results and Discussion. Figure 1 shows the changes in the light transmission of the polymer solutions during temperature scanning (a) and during pressure scanning (b). The midpoint of the change was recorded as the

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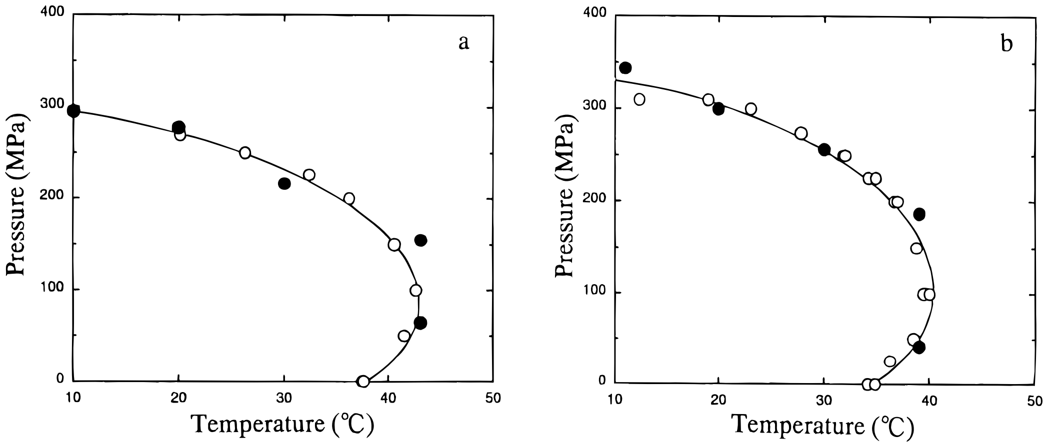

Figure 2. $T-P$ diagrams for the cloud point of PNVIBA (a) and PNIPAM (b) solutions: (a) 0.1 wt/v %; (○) temperature scanning; (●) pressure scanning; (b) 0.5 wt/v %; (○) temperature scanning; (●) pressure scanning.

apparent temperature or pressure of transition. These transitions were measured at various pressures and temperatures, during both increasing scanning and decreasing scanning. Only slight differences were detected in the transition point for either direction of parameter scanning. For the calorimetric measurement of PNIPAM, Tiktopulo *et al.*[10] added a low concentration (<0.03%) of surfactant to reduce intermolecular interactions, but we have observed little apparent effect on the transition temperature by adding SDS at such a low concentration.

The obtained data were plotted in the form of a $T-P$ diagram (Figure 2a). The results for PNVIBA were then compared against PNIPAM in Figure 2b. The data obtained from the T-scanning and P-scanning showed slight differences, especially in the case of PNVIBA, but the overall features of the diagram did not differ significantly.

Near atmospheric pressure, the transition temperature increased with increasing pressure. However, at pressures higher than 150 MPa, it decreased with increasing pressure. Hence, there appeared to be a turning point in the curve for both polymers; PNVIBA showed it at a relatively lower pressure, and the decrease in transition temperature with further increasing pressure was relatively sharp. The results for PNIPAM were essentially similar to those previously reported.[15] These turning points resulted in a characteristic light transmission change when observed at a constant higher temperature with changing pressure (Figure 1c). A turbid solution at lower pressure became clear at medium pressure, but then turned cloudy again at much higher pressures. The process can be reversed freely by decreasing the pressure down to 0.1 MPa.

PNVIBA showed a higher transition temperature at atmospheric pressure, and this can be explained by the fact that hydration to the side groups of this polymer at lower temperatures is stronger than in PNIPAM. In contrast, PNVIBA showed a lower transition pressure at 10−30 °C. This might indicate that the hydration shell of this polymer has a relatively larger volume decrease at elevated pressures.

Hawley introduced a thermodynamic interpretation of the denaturation or absorbance change of some proteins[16] by considering up to the second derivatives of the free energy of transition (eq 1). They predicted the ellipsoid diagram for the pressure−temperature dependence of protein denaturation (eq 2) by assuming that $T \simeq T_0$ ($(T - T_0)/T_0 \ll 1$).

$$\Delta G = \Delta\beta(P - P_0)^2/2 + \Delta\alpha(P - P_0)(T - T_0) - \Delta C_p[T(\ln(T/T_0) - 1) + T_0] + \Delta V_0(P - P_0) - \Delta S_0(T - T_0) + \Delta G_0 \quad (1)$$

$$\Delta G = \Delta\beta(P - P_0)^2/2 + \Delta\alpha(P - P_0)(T - T_0) - [\Delta C_p(T - T_0)^2/2]/T_0 + \Delta V_0(P - P_0) - \Delta S_0(T - T_0) + \Delta G_0 \quad (2)$$

where $\Delta\beta$ and $\Delta\alpha$ are the (absolute) compressibility and the (absolute) expansibility, respectively. Since then, several proteins have been reported to show more or less ellipsoid diagrams. The present data also resulted in ellipsoid diagrams, although a recent discussion on eq 2[17] suggested that the consideration of higher order derivatives of the free energy equation (i.e., compressibility, heat capacity, and/or expansibility are temperature and/or pressure dependent) resulted in distinct deviations from ellipsoidal features. Even if the second derivative of T (the coefficient of ΔC_p) is not approximated as $(T - T_0)^2$, the curve obtained from eq 1 is still almost elliptical. When the center of the ellipsoid is in the first quadrant, the apparent turning point of $\Delta V = 0$ with increasing pressure and of $\Delta S = 0$ with increasing pressure can be observed; in other words, $dP/dT > 0$ near $P - P_0 = 0$ and $T - T_0 = 0$. In the case of proteins, despite the prediction by eq 2, it has been difficult to observe a recovery from the thermally changed structure by increasing the pressure at constant temperature. Only a few studies have reported that the thermal transition point shifted to a higher temperature under mildly high pressures.[18]

The ellipsoid curves drawn in Figure 2 were calculated according to eq 2. The parameters used can only be presented as ratios to the standard free energy of transition, since the transition curve is too sharp to evaluate the independent value of the latter parameter, and an ellipsoid is uniquely determined by these relative values only. The values are compiled in Table 1. The ellipsoid drawn for PNVIBA has a smaller axial ratio (B/A) than PNIPAM, and becomes $\Delta S = 0$ and $\Delta V = 0$ at lower pressures. These pressure levels are determined by $(\Delta C_p \ln(T/T_0) + \Delta S_0)/\Delta\alpha$ (for the point which shows $\Delta S = 0$ or dP/dT becomes infinite) and $(\Delta\alpha(T - T_0) + \Delta V_0)/\Delta\beta$ (for the point which shows $\Delta V = 0$ or dP/dT becomes zero). The dP/dT values at atmospheric pressure are determined by $-(2\Delta C_p(T - T_0) + \Delta S_0)/(\Delta V_0 + \Delta\alpha(T - T_0))$, which is occasionally very similar in these two polymers.

Table 1. Thermodynamic Parameters Calculated from the Results in Figure 2a,b[a]

polymer	$\Delta V_0/\Delta G_0$ (cm^3 cal)	$\Delta S_0/\Delta G_0$ (K^{-1})	$\Delta\beta/\Delta G_0$ (cm^6)	$\Delta\alpha/\Delta G_0$ (cm^3 cal K^{-1})	$\Delta C_p/\Delta G_0$ (K^{-1})
PNVIBA	0.0614	0.0682	−0.002 04	−0.000 0605	0.002 73
PNIPAM	0.0826	0.0703	−0.002 33	−0.000 199	0.004 00

[a] The approximate deviations are about ±8%.

The transition observed here is a change from random coil structure to a globule. The typically observed protein structure change is, however, a process of losing a globular structure and assuming a more random coil-like shape. In this sense, the transition in synthetic polymers resembles the inverse of the so-called cold-denaturation or transition from a random coil-like structure to a disordered globule.[20] The latter transition is also considered to be a precursor of the molten globule.[7] Therefore, the $T-P$ diagrams for proteins versus model polymers show a completely inverted curvature. The parameters in eq 2 that Hawley reported for chymotrypsinogen denaturation had negative signs in ΔV_0, $\Delta\beta$, and ΔS_0 but were positive for ΔG_0, $\Delta\alpha$, and ΔC_p. These are expressed in the direction of the globule to coil transition, and thus the values for the renaturation process of cold denaturation should have inverse signs. Comparing these values for chymotrypsinogen, relative to ΔG_0, against the presently evaluated parameters, it is demonstrated that ΔV_0, $\Delta\beta$, and ΔC_p have opposite signs.

The cold denaturation for real proteins can only be observed using a special kinetic or dynamic approach. Hence, the biomimetic approach introduced here could be complementary to the study of real proteins.

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