"Domain" Coil–Globule Transition in Homopolymers

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ABSTRACT: The temperature-induced coil–globule transition has been studied in dilute aqueous solutions (with 200 mg/L SDS) for different fractions of poly(*N*-isopropylacrylamide) (PNIPAM) and poly(*N*-isopropylmethacrylamide) (PNIPMAM) using scanning microcalorimetry, diffusion, and size-exclusion chromatography (FPLC). It has been shown that both these polymers undergo a coil–globule transition upon temperature increase. This transition is accompanied by cooperative heat absorption and a decrease of heat capacity, which makes it similar to the cold denaturation of globular proteins. The globule–coil transition is an "all-or-none" process only for the fractions with the lowest molecular weights ($\sim 10 \times 10^3$) while fractions of larger molecular weights behave as if they consist of quasi-independent cooperative units, the "domains". The number of "domains" in a macromolecule is proportional to the molecular weight of the polymer. This suggests that the "domain" character of cooperative transitions in large proteins does not, in principle, need evolutionary-selected amino acid sequences but can occur even in homopolymers.

1. Introduction

It is well known that the heat denaturation of *small* proteins is an *all-or-none transition*, i.e., an intramolecular analog of the first-order phase transition. This was established by a comparison of the effective (van't Hoff) value of the denaturation enthalpy ΔH^{eff} (related to a cooperative unit which undergoes a transition as a whole) with its calorimetric value ΔH^{cal} (related to a protein molecule). The coincidence of these two values means that a cooperative unit coincides with a protein molecule, i.e., that a protein molecule denatures as a whole without the coexistence of native and denatured parts in the same molecule.[1,2] However, this is not the case for *large proteins*, whose temperature-induced denaturation has been shown to be *not* an *all-or-none transition*.[3] The absence of all-or-none denaturation of large proteins has been interpreted in terms of their *domain structure*.[3,4]

In fact, in a number of cases the temperature melting of large proteins *can be resolved into two or several relatively independent transitions* corresponding to the melting of different structural domains of these proteins. In some cases the domains can be isolated by splitting of an intact protein molecule and they show the temperature melting approximately under the same conditions as when the domains are incorporated into the intact protein. For example, the "thermodynamic domains" of fibrinogen[5] or plasminogen[6] observed by temperature melting coincide with the structural lobes of a protein molecule.

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However, in some other cases denaturation of an all-or-none character cannot be resolved into two or several transitions which can be ascribed to different structural domains. Examples of such behavior include papain,[7] pepsinogen,[8] and retinol-binding protein.[9] The last example is especially convincing as the retinol-binding protein is arranged as an antiparallel β-barrel which cannot be divided into two lobes. Nevertheless, the van't Hoff enthalpy of its heat denaturation is twice smaller than the calorimetric enthalpy.

To understand the "domain" melting of proteins structurally not consisting of several domains, it is worthwhile to study simple models which can also undergo cooperative changes of their 3D structure. There are some homopolymers including polystyrene,[10–13] poly(*N*-isopropylacrylamide) (PNIPAM),[14–19] poly(*N*-isopropylmethacrylamide) (PNIPMAM),[15,20] poly(*N*-*n*-propylacrylamide), and poly(*N*-cyclopropylacrylamide)[21] which undergo coil–globule transitions induced by temperature. However, the extent of cooperativity of these transitions remains open. The study of these transitions may help to understand whether the "domain" melting of proteins is determined by the unique character of their sequences or whether it is a typical feature of other large macromolecules.

We have recently studied[22] the thermodynamic parameters of the coil–globule transition for PNIPAM of high molecular weight (7000×10^3) which was shown previously[19] to undergo such transition upon heating in a very narrow interval of temperature. The calorimetric enthalpy value for this polymer is more than 100-fold greater than the van't Hoff enthalpy value, which clearly shows the absence of an all-or-none transition. According to the conventional protein terminology, it can be concluded that this polymer consists of more than 100 "thermodynamic domains".

To study further this unusual behavior, we have investigted the thermodynamic parameters of temperature-induced coil–globule transitions for five fractions of PNIPAM (with molecular weights from 11 000 to 370 000) and for three fractions of PNIPMAM (molec-

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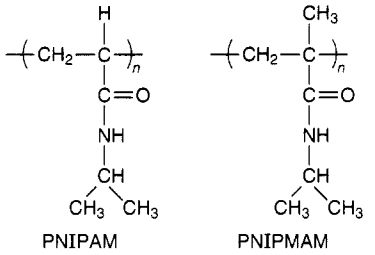
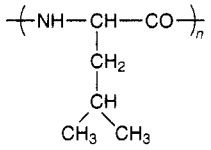
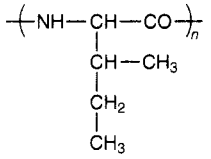
ular weights from 13 000 to 83 000). PNIPAM and PNIPMAM are simple vinyl homopolymers differing by the presence of one methyl group attached to a vinyl backbone:



From the chemical point of view, these polymers are homologous to polypeptides: PNIPAM is homologous to poly(leucine)



while PNIPMAM chemically differs from poly(isoleucine)



only by one additional CH_2 group in each monomer unit. However, an important difference between these vinyl polymers and polypeptides is that vinyl polymers have a nonpolar backbone and include peptide groups in their side chains, while poly(leucine) and poly(isoleucine) include peptide groups in their backbone and have entirely nonpolar side chains.

2. Materials and Methods

2.1. Preparation of Polymers. PNIPAM and PNIPMAM were obtained by free-radical polymerization of the corresponding monomers in dimethylformamide. Azobis(isobutyronitrile) (AIBN) was used as an initiating agent. The monomers were polymerized for 20–22 h at 60 °C in argon-filled glass ampules. The polymers were precipitated from the reaction mixture by diethyl ether and then purified by threefold reprecipitation from a methanol solution by the same ether. The polymers were vacuum dried to constant weight at room temperature. The amounts of the solvent and initiating agent in the reaction mixture were varied to obtain polymers of different molecular weights (the monomer:solvent weight ratio was varied from 1:0.5 to 1:2 and the initiating agent concentration was varied from 1.4 to 0.11% of the weight of monomer participating in the reaction).

Polymers were fractionated by precipitation from ethanol solutions at concentrations from 5 to 10% with diethyl ether as a precipitating agent. The molecular weights of the fractions (M) were determined from the values of intrinsic viscosity measured in methanol and in water,[23] and these M values are presented in Tables 1–3. Similar values have also been obtained for some fractions with light scattering in methanol using a Sofica photogoniodiffusometer (France). The polydispersity of the two PNIPAM fractions with the highest molecular weights has been estimated as $M_w/M_n = 1.4–1.6$, where M_w and M_n are the weight- and number-average molecular weights, respectively. M_w/M_n has been measured by sedimentation in methanol at 45 000 rpm using a MOM 3180 ultracentrifuge (Hungary) by sedimentation curve analysis.[24]

2.2. Preparation of Polymer Solutions. We used aqueous solutions of polymers containing 200 mg/L sodium dodecyl sulfate (SDS) as in our previous studies.[22] Prior to measurements all the homopolymer samples were dissolved in appropriate SDS solutions and stored for 15–20 h in a refrigerator to complete dissolution of the polymer.

Polymer concentrations were 0.2–0.8 mg/mL for calorimetric measurements, about 0.01 mg/mL for FPLC experiments, and 0.1–0.3 mg/mL for diffusion measurements.

2.3. Calorimetric Measurements. Calorimetric studies were performed on a precision capillary scanning microcalorimeter DASM-4A (Russia) at an excess pressure of 5.07×10^5 Pa. The calorimetric cell volume was 0.5 mL. The heating rates were 1, 0.5, 0.125, and 0.064 K/min.

2.4. Size-Exclusion Chromatography. A stock solution of polymer was loaded on a Superose-12 column equilibrated with appropriate SDS solutions. The flow rate was 15 mL/h. The elution profiles were obtained using a 2158 Uvicord SD instrument (LKB, Sweden). FPLC measurements were done at two temperatures, 23 and 50 °C. The column temperature was maintained (±0.5 °C) by housing the column in a glass jacket attached to a MLW U4 circulating water bath (VEB MLW, Germany) which also held the solvent reservoir and protein samples.

The Stokes radii (R_s) of the polymer molecules were measured by FPLC on a Superose-12 column calibrated by native and unfolded proteins with known Stokes radii.[25] The R_s value for a polymer under the given conditions was calculated from the respective elution volume (V^{el}) value according to the equation[25]

$$R_s = \frac{(1000/V^{el}) - 52.1}{0.725} \quad (1)$$

The Stokes radii of protein molecules of a given molecular weight M in native (R_s^N) and completely unfolded (R_s^U) states were estimated from the empirical equations,[25] based on the experimental data of Tanford:[26]

$$\log(R_s^N) = 0.369 \log(M) - 0.254 \quad (2)$$

and

$$\log(R_s^U) = 0.533 \log(M) - 0.682 \quad (3)$$

2.5. Diffusion Measurements. Diffusion coefficients D were measured by macroscopic diffusion using a polarizing interferometer.[27] In some cases this method is more favorable than other ones. It uses rather small concentrations (0.1–0.3 mg/mL), and, what is more important, interferometric optics allows one to observe the distribution of the concentration gradient dc/dx in the diffusion cell at different moments of the experiment. In the case of a monodisperse system, the dc/dx distribution is described by a Gaussian curve. In the case of a polydisperse system, the experimental curve is a superposition of different Gaussian curves which correspond to the individual components.[24] Several approaches have been proposed for a practical calculation of the diffusion coefficients of the individual components.[28–30] Diffusion coefficients were measured for PNIPAM fractions with molecular weights of 11 200 and 160 000 at 23 °C (for coils) and at the transition temperature.

The partial specific volumes of PNIPAM and PNIPMAM determined with an Anton-PAAR density meter (Austria) at 25 °C were 0.870 and 0.850 cm^3/g, respectively.

PNIPAM and PNIPMAM fractions containing luminescent labels of anthracene structure (1 label per 1000 monomer units) were used for fluorescence measurements.[20] In this study we investigate unlabeled and labeled polymers. Since unlabeled polymer fractions have a much stronger tendency

Table 1. Stokes Radii of Low Molecular Weight Fractions of PNIPAM and PNIPMAM at Low and High Temperature As Compared with Those Calculated for Globular Proteins of Corresponding Molecular Weights

		Stokes radius value (Å)			
polymer	mol wt $10^{-3}M$	polymer at 23 °C[a]	unfolded protein 6 M GdmCl[b]	polymer at 50 °C[a]	native protein[a]
PNIPAM	11.2	33 ± 3	30 ± 2	16 ± 2	17 ± 1
	27.4	46 ± 4	48 ± 2	21 ± 2	24 ± 1
PNIPMAM	13.0	37 ± 3	32 ± 2	16 ± 2	18 ± 1

[a] Calculated from V^{el} by eq 1. [b] Calculated from M by eq 2:[25] $\log(R_s^N) = 0.369 \log(M) - 0.254$. [c] Calculated from M by eq 3:[25] $\log(R_s^U) = 0.533 \log(M) - 0.682$.

to aggregation than the labeled ones, we present and discuss mainly the experimental data for labeled polymers.

3. Results and Discussion

3.1. Coil–Globule Transition in PNIPAM and PNIPMAM. Both PNIPAM and PNIPMAM have a tendency to aggregate in water at elevated temperature, which hinders the observation of the coil–globule transition in these polymers. At high molecular weights this aggregation can be prevented or at least largely reduced by the addition of sodium dodecyl sulfate (SDS) in small concentrations.[16,19,31] For such a case (in the presence of 200 mg/L of SDS), the coil–globule transition in a very high molecular weight fraction of PNIPAM has been well established.[19] Here we present data which demonstrate a similar transition in low molecular weight fractions of PNIPAM and PNIPMAM prepared and fractionated under other conditions. In this case the addition of SDS does not substantially reduce aggregation, but we still used solutions with 200 mg/L of SDS to facilitate comparison of the results with our previous data for the high molecular weight fraction of PNIPAM (see ref 22).

Figure 1 presents the elution profiles obtained at 23 and 50 °C for the fractions with the lowest molecular weights of 11.2×10^3 and 27.4×10^3 for PNIPAM and 13.0×10^3 for PNIPMAM. Since the Superose-12 column used in these experiments can be applied only to small molecules (with $M < 30 \times 10^3$ for globules), it was impossible to investigate higher molecular weight fractions using this column.

The elution profiles of the studied polymer fractions are shown in Figure 1. The left peak in panels A and B of Figure 1 does not depend on the polymer molecular weight and coincides with the column void volume. Therefore this peak can be attributed to large aggregates. An increase of temperature results in enhancement of this peak (or to its appearance in the case of PNIPMAM), which suggests an increase of polymer aggregation. The right peak shifts to large elution volume at an increase of temperature, reflecting the decrease of polymer dimensions.

The right peak at both 23 and 50 °C is broader than the peaks usually obtained for proteins with a Superose-12 column. This can result from both molecular weight heterogeneity and small association. The position of this peak depends on the polymer molecular weight and on temperature. Calibration of the Superose-12 column permits estimation of the Stokes radii, whose values for the three studied fractions are shown in Table 1. These values can be considered only as rather crude estimates, since the elution volume of a macromolecule depends not only on its dimensions but also on its interaction with the column matrix. Consequently, the calibration performed for proteins may be not strictly valid for synthetic polymers. Nevertheless, the resulting R_s's for 23 and 50 °C are close to those obtained for the native

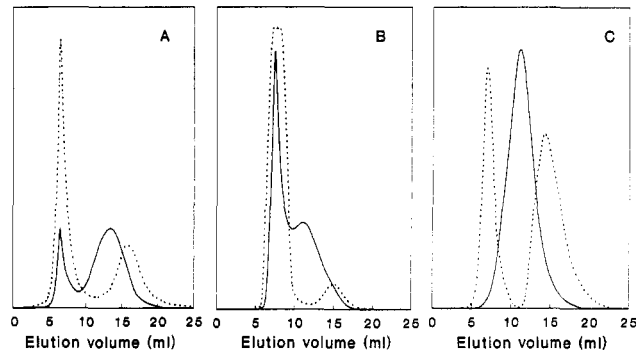


Figure 1. FPLC profiles of polymer fractions at 23 °C (solid curves) and at 50 °C (dotted curves): (A) PNIPAM, $M = 11.2 \times 10^3$; (B) PNIPAM, $M = 27.4 \times 10^3$; (C) PNIPMAM, $M = 13.0 \times 10^3$.

(globular) and unfolded proteins of the corresponding molecular weight, respectively (see Table 1). This confirms that the low molecular weight fractions of PNIPAM and PNIPMAM studied in this paper undergo a temperature-induced coil–globule transition.

The data on the temperature-induced coil–globule transition in these polymers was also checked by measurements of diffusion coefficients for PNIPAM fractions with M = 11 200 and 160 000 by the interferometric method.[27] This procedure was applied previously to determine protein dimensions in the native, molten globule, and unfolded states.[32] The tendency of polymers to aggregate hampers these measurements; however, diffusion coefficients of individual components can be obtained from an experimental curve if the differences of these diffusion coefficients are significant.[28–30]

Diffusion measurements show the presence of fast and slow components for both the 11 200 and 160 000 fractions, which can be attributed to monomeric and associated states of polymers, respectively (it is known that macromolecules with the highest diffusion coefficient, i.e., with the lowest molecular weight, are the first to diffuse, ahead of the other macromolecules[24]). This fact is in accordance with the results obtained from FPLC measurements. Estimation of the diffusion coefficient $D_{20,w}$ of the fast component for the 11 200 fraction of PNIPAM leads to values of 8.8×10^{-7} and 13.0×10^{-7} cm²/s for 23 °C and the transition temperature, respectively, where $D_{20,w}$ is the D value at a given temperature T reduced to the viscosity of water at 20 °C. These values are typical for unfolded and globular proteins with molecular weights of ~10 000. This confirms the temperature-induced coil–globule transition for the fast component of this fraction. The Stokes radii R_s were calculated using the equation $D = kT/6\pi\eta_0 R_s$, where η_0 is the viscosity of the solvent and k is Boltzmann's constant. The above-mentioned values of the diffusion coefficients correspond to R_s = 24 and 17 Å, respectively. Both these values do not differ greatly

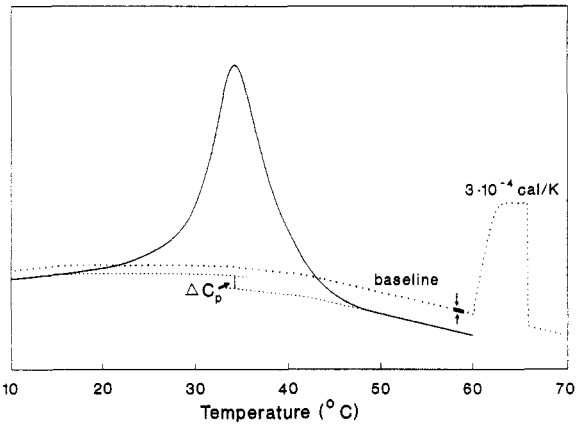


Figure 2. Heat capacity recording of PNIPAM in 200 mg/L SDS, $c = 0.8$ mg/mL, $M = 27.4 \times 10^3$. The base line with a calibrating energy pulse was obtained for a solvent of 200 mg/L SDS in water. Arrows show experimental errors of a calorimetric recording which does not exceed 2×10^{-6} cal/K. Extrapolations of differences between heat capacities of solvent and solution from low and high temperatures are shown by dashed lines. ΔC_p is the jump of heat capacity upon the coil–globule transition.

from those estimated by FPLC. It would be difficult to expect a closer coincidence, since the diffusion of semipermeable coils is much more complicated than that of rigid spheres and may be different on a column and in a solution. The results of diffusion measurements confirm a substantial decrease in the dimensions of the 11 200 PNIPAM fraction upon temperature increase. Qualitatively, similar results have been obtained for the 160 000 fraction of PNIPAM: $D_{20,w} = 1.8 \times 10^{-7}$ cm²/s for the coil-like state and 4.3×10^{-7} cm²/s for the globular state, which give $R_s = 120$ and 50 Å, respectively.

3.2. Calorimetric Evidence for a Compact Structure of PNIPAM and PNIPMAM at High Temperature. The heat capacity of a native (compact) protein molecule is essentially lower than that of unfolded protein chains and other denatured states.[1] In other words, the melting of proteins is accompanied by an increase of their heat capacity at high temperatures for heat denaturation and at low temperatures for cold denaturation. Respectively, renaturation of a protein upon heating after cold denaturation leads to the decrease of its heat capacity[33,34] as compared with that in the denatured state. We have observed a similar behavior for a PNIPAM fraction with a high molecular weight.[22]

Figure 2 represents a recording of the heat capacity $C_p(T)$ *versus* temperature for an aqueous solution of PNIPAM ($M = 27.4 \times 10^3$). The "base line" (i.e., the heat capacity of buffer solution) with a calibrated mark is also shown for comparison. It is seen that the difference between the heat capacities of solution and solvent is substantially more negative at high temperatures than that at low ones. The extrapolation of heat capacity curves from low and high temperatures to the middle of transition permits (see Figure 2) evaluation of their difference, ΔC_p.

The same picture (lower values of excess heat capacity at high temperatures) was observed for all other studied fractions of both PNIPAM and PNIPMAM (see Figure 3). The increase of heat capacity is believed to be the result of the exposure of nonpolar groups to water.[35] Hence, lower $C_p(T)$ values at an elevated temperature suggest that both PNIPAM and PNIPMAM are more

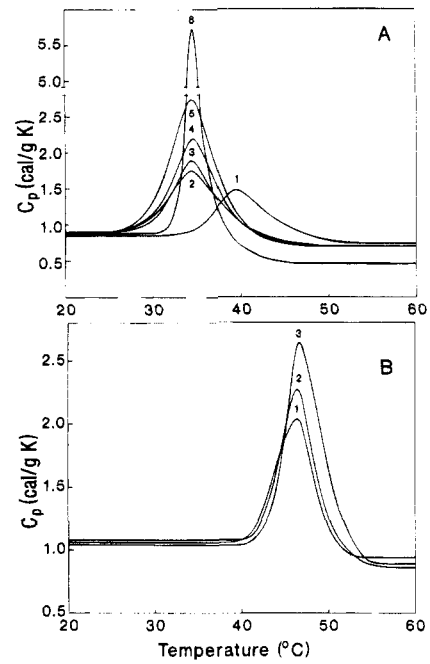


Figure 3. Dependence of partial specific heat capacity on temperature: (A) PNIPAM fractions with molecular weights (1) 11.2×10^3, (2) 27.4×10^3, (3) 59.0×10^3, (4) 160.0×10^3, and (5) 370.0×10^3; (B) PNIPMAM fractions with molecular weights (1) 13.0×10^3, (2) 56.0×10^3, and (3) 83.0×10^3. Curve 6 in (A) refers to the high molecular weight (7000×10^3) fraction of PNIPAM studied earlier.[22]

compact at higher temperatures. This is confirmed by FPLC and diffusion data and permits extension of these results to other studied fractions.

3.3. Calorimetric Studies of the Heat-Induced Coil–Globule Transition in PNIPAM and PNIPMAM. Figure 3 presents the temperature dependence of the partial specific heat capacity of all the studied fractions of PNIPAM (Figure 3A) and PNIPMAM (Figure 3B). Unlike the situation observed earlier[22] for the fraction of PNIPAM with a very high molecular weight ($M = 7000 \times 10^3$), in this case a 15-fold decrease in the heating rate (from 1 to 0.064 K/min) does not significantly affect the $C_p(T)$ curves. This means that equilibrium thermodynamics can be directly applied to these experiments. A repeated heating of each polymer demonstrates the complete reversibility of the heat absorption curves. The data presented in Figure 3 show that the coil–globule transitions in both PNIPAM and PNIPMAM occur in narrow temperature ranges. The small asymmetry of the heat absorption peak may be due to the heterogeneity related either to the intrinsic molecular weight distribution or to association. However, this asymmetry in all cases is not significant and does not impede a simple thermodynamic analysis of the heat absorption curves. Moreover, there is no concentration dependence of the partial heat capacity (i.e., the heat of the transition Q; see below) in the used concentration range (0.2–0.8 mg/mL).

Figure 3 and Tables 2 and 3 demonstrate that the transition temperature for both polymers virtually does not depend on their molecular weight. The only exception is the fraction of PNIPAM with $M = 11\,200$, where the transition temperature is a little higher. The heat capacity jump between the coil-like and globule-like states in both polymers also does not depend on their molecular weights. A comparison of the transition temperature in PNIPAM and PNIPMAM demonstrates that the presence of a methyl group in the PNIPMAM

Table 2. Thermodynamic Parameters of the Coil–Globule Transition in Poly(*N*-isopropylacrylamide)

polym mol wt, $10^{-3}M$	T_m (°C)	ΔH^{cal} (kcal/mol)	ΔH^{eff} (kcal/mol)	$\Delta C_p(T_m)$ (cal/(g·K))	n^a	no. of monomer residues in a "domain"
11.2	39.3	80	78	0.20	1.0	99
27.4	34.2	210	80	0.21	2.6	93
59.0	34.5	400	72	0.22	5.6	93
160.0	34.5	1500	95	0.21	16.0	89
370.0	34.3	3600	93	0.23	39.0	85
av value			84 ± 10	0.21 ± 0.01		92 ± 4
7000[b]	34.3	105 000	810	0.44	120.0	600

[a] $n = \Delta H^{cal}/\Delta H^{eff}$ = number of cooperative units ("domains"). [b] Reference 22.

Table 3. Thermodynamic Parameters of the Coil–Globule Transition in Poly(*N*-isopropylmethacrylamide)

polym mol wt, $10^{-3}M$	T_m (°C)	ΔH^{cal} (kcal/mol)	ΔH^{eff} (kcal/mol)	$\Delta C_p(T_m)$ (cal/(g·K))	n^a	no. of monomer residues in a "domain"
13.0	47.0	125	140	0.13	0.9	125
32.0	46.8	270	132	0.13	2.0	118
83.0	46.8	590	132	0.13	4.4	117
av value			135 ± 3	0.13 ± 0.01		120 ± 4

[a] $n = \Delta H^{cal}/\Delta H^{eff}$ = number of cooperative units ("domains").

monomer increases the temperature of the coil–globule transition, i.e., destabilizes the globular state relative to the coiled one.

To determine the cooperativity of these transitions, the effective enthalpies of transition (ΔH^{eff}) must be compared with their real (calorimetric) values (ΔH^{cal}). Both these enthalpies were estimated according to the well-known equations (see, e.g., ref 1, 3, and 22):

$$\Delta H^{eff} = 4RT^2\frac{C_p(T_m)}{Q_{tr}} \quad (4)$$

and

$$\Delta H^{cal} = MQ_{tr} \quad (5)$$

where $C_p(T_m)$ is the specific heat capacity of a macromolecule at temperature T_m of the heat absorption maximum, Q_{tr} is the heat of the transition reaction normalized to 1 g of the solute (the area under the heat absorption peak), and M is the molecular weight.

The thermodynamic parameters obtained for PNIPAM and PNIPMAM are presented in Tables 2 and 3, respectively. The tables show that the values of the molar calorimetric enthalpy increase with increasing molecular weight. These results on the molecular weight dependence of the calorimetric enthalpy are also presented in Figure 4.

On the other hand, the effective (van't Hoff) enthalpy for both polymers virtually does not depend on the molecular weight (Figure 5). Since the values of ΔH^{eff} determine the dimensions of a "cooperative unit", which undergoes a globule–coil transition as a whole, the comparison of ΔH^{eff} with ΔH^{cal} permits estimation of the number of cooperative units in a given polymer fraction: $n = \Delta H^{cal}/\Delta H^{eff}$. Figure 6 presents the molecular weight dependence of n, showing that the number of cooperative units ("domains") is proportional to the molecular weight.

In the terminology used for the description of protein denaturation (see, e.g., refs 1 and 2), the coil–globule transition in both PNIPAM and PNIPMAM occurs according to a "domain" mechanism (the independent formation of "domains" or coorperative units). "Domains" of PNIPAM and PNIPMAM contain ~90 and ~120 monomer residues, respectively (see Tables 2 and 3). The molecular weights of these "domains" are ~10 000 for PNIPAM and ~13 000 for PNIPMAM. Consequently, only the fractions of both polymers with the lowest molecular weight (11.2×10^3 for PNIPAM and 13.0×10^3 for PNIPMAM) undergo the "all-or-none" transition with $\Delta H^{cal}/\Delta H^{eff} \cong 1$. All the other fractions behave as if they consist of quasi-independent "domains" with molecular weights of $(10-15) \times 10^3$.

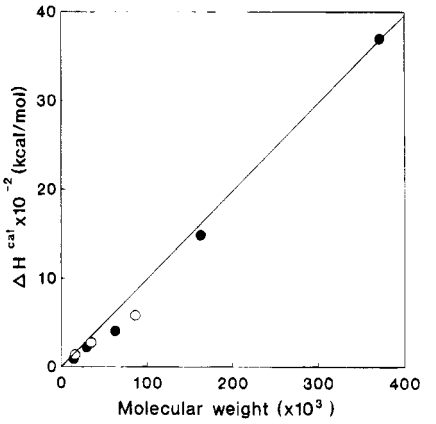


Figure 4. Molecular weight dependences of transition enthalpy ΔH^{cal} for PNIPAM (filled circles) and PNIPMAM (open circles).

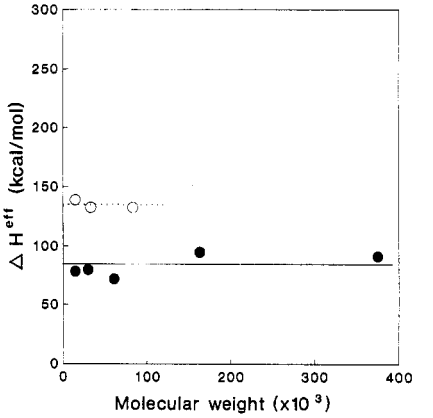


Figure 5. Molecular weight dependence of effective enthalpy ΔH^{eff} of transition for PNIPAM (filled circles) and PNIPMAM (open circles).

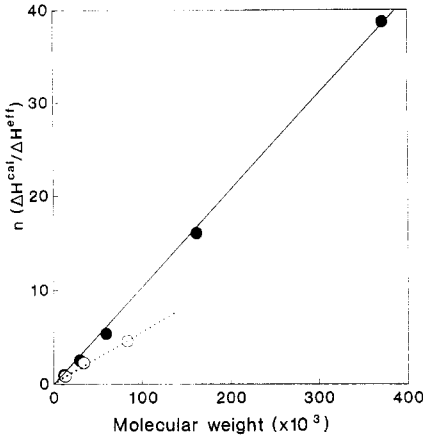


Figure 6. Molecular weight dependences of the number of "domains" n ($n = \Delta H^{cal}/\Delta H^{eff}$) for PNIPAM (filled circles) and PNIPMAM (open circles).

The character of the temperature transition for the PNIPAM and PNIPMAM fractions is similar to that of the transition in a high molecular weight sample ($M = 7000 \times 10^3$) of PNIPAM (see ref 22), and these results are compared in Table 2 and Figure 3A. Table 2 shows that the 7000×10^3 PNIPAM fraction has the same transition temperature as the low molecular weight ones and that its calorimetric enthalpy coincides within 20% with the value extrapolated from the molecular weight dependence for low molecular weight fractions (not shown). On the other hand, the effective enthalpy value for the 7000×10^3 fraction is about an order of magnitude higher than that obtained in the present paper for other PNIPAM fractions (see Table 2). Up to the present, there is no convincing explanation of this discrepancy, which, however, does not alter the main conclusions of this study. It should be mentioned that the highest molecular weight sample[22] has been obtained by polymerization and fractionation substantially different from the procedures used here.

3.4. Aggregation and Calorimetric Measurements. It is known that calorimetric measurements can be affected by aggregation. In the case of strong aggregation, a sharp drop of the heat capacity is usually observed just after the heat absorption peak. This is not the case for the $C_p(T)$ curves for all the studied PNIPAM and PNIPMAM fractions. Moreover, Figures 4 and 5 clearly show that the thermodynamic parameters of melting of all the fractions follow the same molecular weight dependences despite the remarkable differences in their aggregation. This suggests that the aggregation of polymer fractions detected by FPLC and diffusion measurements does not influence very much the measured values of the thermodynamic parameters.

4. Conclusions

From the data presented in this paper, a conclusion can be drawn that both the studied homopolymers undergo a globule−coil transition induced by a temperature decrease. This transition occurs as a quasi-independent disruption of the cooperative units ("domains") whose number is proportional to the molecular weight of a polymer. According to the "all-or-none" mechanism typical of small globular proteins, the coil−globule transition occurs only for fractions with the lowest molecular weight, similar to that of one "domain". This behavior described here for *homopolymers* is similar to that observed earlier for heat[3] and "cold" (see ref 36) denaturation of proteins consisting of several "domains". These results suggest that the "domain" character of denaturation of large proteins does not necessarily need evolutionary-selected amino acid sequences, but it rather can be typical of other large macromolecules, including even homopolymers.

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