Cooperativity of the Coil–Globule Transition in a Homopolymer: Microcalorimetric Study of Poly(*N*-isopropylacrylamide)

E. I. Tiktopulo,*,† V. E. Bychkova,† J. Rička,‡ and O. B. Ptitsyn†,§

Institute of Protein Research, Russian Academy of Sciences, 142292 Pushchino, Moscow Region, Russia, Institute of Applied Physics, University of Berne, 3012 Berne, Switzerland, and Laboratory of Mathematical Biology, National Cancer Institute, NIH, WSC, Bethesda, Maryland 20892

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ABSTRACT: The temperature-induced intramolecular coil–globule transition in poly(*N*-isopropylacrylamide) has been studied by microcalorimetry to investigate the cooperativity of this transition. Measurements were performed in the presence of sodium dodecyl sulfate (SDS) which prevents the polymer from aggregation both in the coil and in the globule state. It has been shown that the effective (van't Hoff) enthalpy of transition of a cooperative unit is 120 times less than the calorimetric enthalpy of a polymer molecule. This means that a coil–globule transition in this polymer is not an "all-or-none" process and occurs independently in cooperative units ("domains") which are 120 times smaller than the polymer molecule and have a molecular weight about 6×10^4.

Introduction

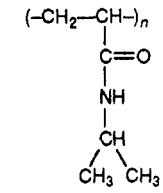
The conformation of macromolecules may exhibit a remarkably strong dependence on the solvent and other conditions, e.g. the temperature. An example of utmost fundamental importance is the denaturation of proteins where even a minor change of the environment may result in an abrupt loss of the biological activity and a dramatic change of the structure. One of the pertinent problems concerning such conformational transitions is their degree of cooperativity.

It is well-known[1] that the denaturation of small proteins is an "all-or-none" process; i.e. the protein molecule undergoes this transition as a whole. On the other hand, the denaturation of larger proteins is not an "all-or-none" process and proceeds through a set of intermediates consisting of several native and denatured parts of molecules (domains), each of them denatures as a whole.[2,3] These "melting" domains may coincide with the structural lobes of a protein molecule, but even some of the proteins that consist of a single structural lobe melt as independent "melting" domains.

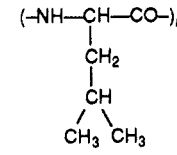
To understand the physical reasons for this difference in the character of denaturation of small and large proteins is worthwhile to compare them with globule–coil transitions in synthetic models, including the simplest case of homopolymers. Since the first experimental evidence in the early seventies,[4] these transitions have been extensively studied by various techniques and for a number of homopolymers including poly(styrene), poly(*N*-isopropylacrylamide), and poly(*N*-isopropylmethylacrylamide).[5–15] Most of these studies, however, concentrated on the determination of molecular parameters (e.g. molecular dimensions and intramolecular dynamics) in the initial and final states, without addressing the pertinent question of the degree of cooperativity.

The best way to study cooperativity of the temperature-induced transition is to use microcalorimetry[1] which permits the determination of the dimensions of a "cooperative unit" (which undergoes the transition as a whole) and the comparison of it with the dimensions of the protein molecule. In the present study we therefore apply this technique to the study of cooperativity of the temperature-induced coil–globule transition in poly(*N*-isopropylacrylamide).

Poly(*N*-isopropylacrylamide) (poly(NIPAM))



is a polyvinyl polymer which is a chemical isomer of poly(leucine)



but has a polar peptide group in the side chains rather than in the backbone. Poly(NIPAM) in water solutions has been studied by a number of authors.[8–10,12,13] At room temperature poly(NIPAM) is dissolved in the form of coils but undergoes a coil–globule transition when the temperature is slightly elevated. In pure water this intramolecular collapse of poly(NIPAM) is accompanied by intermolecular aggregation. However, the aggregation can be prevented by small concentrations of sodium dodecyl sulfate (SDS) without disturbing appreciably the globule state,[10,12,13] and this allows the study of the coil–globule transitions at the molecular level.

In the present microcalorimetric study we use a poly(NIPAM) sample with a molecular weight of 7×10^6 which has been extensively studied previously[13] by static and dynamic light scattering. The collapse from the statistical coil to the globular state takes place at 34 °C for SDS concentrations ranging from 150 to 250 mg/L. (Above 300 mg/L the surfactant forms polymer-induced micelles and the transition temperature increases.) The collapse leads to a drastic decrease of the radius of gyration R_g from 1350 to 160 Å, i.e. about 8.5 times. The hydrodynamic

* To whom correspondence should be addressed.
† Russian Academy of Sciences.
‡ University of Berne.
§ NIH.
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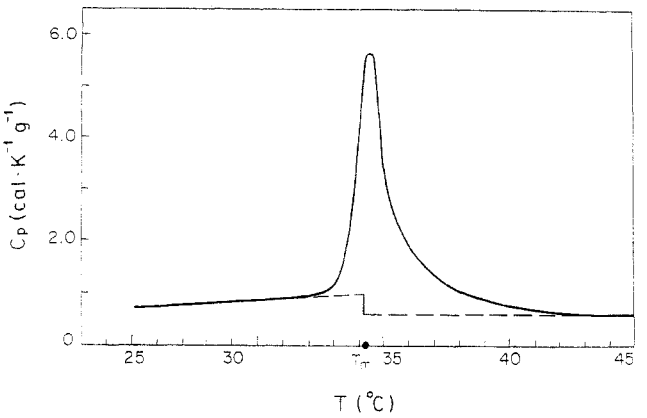

Figure 1. Temperture dependence of the partial specific heat capacity of poly(*N*-isopropylacrylamide) in a water solution in the presence of SDS (200 mg/L). The heating rate is 1 K/min. Dashed lines show the extrapolations of heat capacity curves from low and high temperatures (i.e. from the coil and globular states of the polymer) to the middle of the transition region. The short vertical line shows $\Delta C_p(T_m)$, i.e. the difference between the heat capacities of the globular and the coil states at the middle of transition (T_m).

radius R_s drops from 1010 to 198 Å. The ratio R_g/R_s, reflecting the shape of the molecule, changes from 1.34 (which is near values expected for the statistical coil) to 0.81 (which practically coincides with the theoretical value for rigid spherical particles). Such a well characterized sample, investigated now at the same conditions as in the light scattering study, guarantees a reliable determination of the degree of cooperativity.

The cooperativity of temperature-induced transitions can be most straightforwardly determined by scanning microcalorimetry. The interpretation of the calorimetric data is based on a phenomenological model of the transition,[1] in which a macromolecule is viewed as consisting of independent "cooperative units" and the transition in each of these units is regarded as a temperature-induced all-or-none transition from one state (e.g. coil state of the cooperative unit) to another state (e.g. globule state of the cooperative unit).

As a cooperative unit by definition undergoes the all-or-none transition, its equilibrium constant is $K = \theta/(1-\theta)$, where θ is the number of moles of cooperative units in one of the two states (in the coil or globule states).

From the temperature dependence of K^{eff} one can calculate the effective enthalpy ΔH^{eff} of the reaction

$$\frac{d \ln K}{dT} \equiv \frac{dH^{eff}}{RT^2} = \frac{1}{\theta(1-\theta)} \frac{d\theta}{dT} \quad (1)$$

Here ΔH^{eff} represents the heat absorbed (or generated) due to conversion of 1 mol of cooperative units.

In calorimetric measurements $\theta(T) = Q(T)/Q_{tr}$, where $Q(T)$ is the heat absorbed at a given temperature, while Q_{tr} is the whole heat of the transition reaction. Therefore $d\theta/dT = (dQ(T)/dT)/Q_{tr} = C_p(T)/Q_{tr}$, ($C_p(T)$ being the specific heat capacity of macromolecules) and

$$\Delta H^{eff} = \frac{RT^2}{\theta(1-\theta)} \frac{C_p(T)}{Q_{tr}} \quad (2)$$

A good estimate of ΔH^{eff} can be obtained using the height of the absorption peak in $C_p(T)$ (see Figure 1). In the middle of the transition ($T = T_m$ where T_m is the temperature at which the maximum occurs) $\theta = 1/2$ and

$$\Delta H^{eff} = 4RT^2 \frac{C_p(T_m)}{Q_{tr}} \quad (3)$$

(Note that the quantity $Q_{tr}/C_p(T_m) = \Delta T$ can be regarded as the effective temperature interval of the transition. The larger the cooperative unit, the sharper is the peak in $C_p(T)$, i.e. the narrower is ΔT.)

On the other hand, from the area of the heat absorption peak one can also determine the "real" calorimetric enthalpy ΔH^{cal} associated with the conversion of 1 mol of the whole polymer molecule as

$$\Delta H^{cal} = M \cdot Q_{tr} \quad (4)$$

where M is the molecular weight and Q_{tr} is the heat of transition referred to 1 g of polymer; i.e. therefore one can determine the molar enthalpy of the coil–globule state transition in the polymer.

To determine the cooperativity of transition in a quantitative way we have to compare the molecular weight of a cooperative unit (i.e. the part of a molecule which undergoes the transition as a whole) with the molecular weight of the whole molecule. To this end we can compare the transition enthalpy of a cooperative unit (which can be determined from the temperature interval of the transition) with the transition enthalpy of a macromolecule which can be determined by microcalorimetry.[1,16] The advantage of scanning microcalorimetry consists of the fact that both quantities, ΔH^{eff} and ΔH^{cal}, can be determined in a single experiment.

In the case of an all-or-none transition the cooperative unit encompasses the whole molecule and therefore the effective enthalpy ΔH^{eff} equals the real enthalpy ΔH^{cal}. The ratio $\Delta H^{cal}/\Delta H^{eff}$ represents an effective number of cooperative units per polymer molecule and thus provides a quantitative measure of the degree of cooperativity.

Experimental Section

The preparation and characterization of poly(NIPAM) has been described previously.[13] Atactic poly(NIPAM) was synthesized by the radical polymerization method to obtain a high molecular weight without essential branching. The polymerization product (yield about 60%) was fractionated in methanolic solutions into seven fractions by means of a Knauer HPLC apparatus and the highest molecular weight fraction has been previously used for the light scattering measurements.[13] We have used the same fraction with the molecular weight $M_w = 7 \times 10^6$. Polydispersity was estimated from quasielastic light scattering in the globular state at the highest scattering angle. The upper limit of M_w/M_n is 1.3. Polymer and surfactant (SDS) stock solutions were mixed, and after 12 h of cooling in a refrigerator the solution was centrifuged for 10 min at 16 000*g*. The polymer concentration was 0.4–0.5 mg/mL. The surfactant concentration was 200 mg/L.

Calorimetric measurements were performed on a precision scanning microcalorimeter DASM-4A (see ref 17) with a cell volume of 0.5 mL at an excess pressure of 5.1×10^5 Pa. Heating rates were 1.0, 0.5, 0.125, and 0.064 K/min.

The dimensions and molecular weight of polymer were checked additionally by measurements of the diffusion coefficient and static light scattering. They completely coincide with those published before in ref 13.

Results

Figure 1 shows the temperature dependence of the partial heat capacity C_p of poly(NIPAM) in water solution in the presence of SDS (200 mg/L). The figure demonstrates that the coil–globule transition in poly(NIPAM)

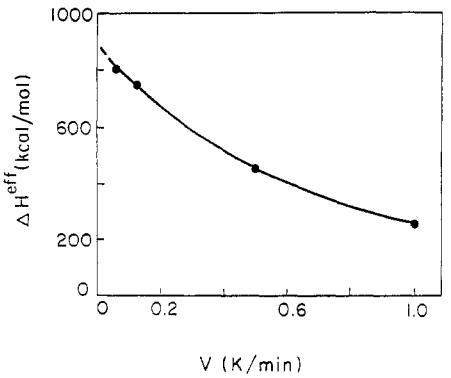

Figure 2. The dependence of effective (van't Hoff) enthalpy of transition ΔH^{eff} on the heating rate V.

Table 1. Calorimetric Parameters of the Coil–Globule Transition of Poly(*N*-isopropylacrylamide)

rate of heating (K/min)	T_m (°C)	$\Delta C_p(T_m)$ [cal/(g·K)]	ΔH^{cal} (kcal/mol)	ΔH^{eff} (kcal/mol)
1	34.3	0.44	104 000	252
0.5	34.2	0.44	107 000	437
0.125	34.2	0.45	104 000	750
0.064	34.3	0.43	100 000	810

is *endothermic* with a sharp heat capacity peak. The temperature of the maximum heat capacity is 34.3 ± 0.1 °C and does not depend on the heating rate (see below).

The difference between the heat capacities of the coil and the globular states can be measured as the difference between $C_p(T)$ curves extrapolated from both sides of the transition (i.e. from the coil and the globular states) to its middle point (see Figure 1). It is clearly seen in this figure that the heat capacity of poly(NIPAM) at high temperatures is *smaller* than that at low temperatures. This decrease of heat capacity at high temperatures after transition is just opposite the usual behavior of $C_p(T)$ during temperature denaturation of globular proteins which is accompanied by partial unfolding of the chain. The main reason of the increase of heat capacity due to unfolding of proteins is believed to be the exposure of nonpolar groups to water (see e.g. ref 16). Correspondingly, the present decrease of $C_p(T)$ reflects the collapse of the polymer upon heating. The collapse causes a decrease of the number of polymer–water contacts in analogy with refolding of a protein upon heating after cold denaturation.[18]

The interpretation of the data in terms of eqs 1–4 assumes equilibrium thermodynamics and we must therefore be sure that the process is reversible and that its thermodynamic parameters do not depend on the heating rate. The reversibility of the temperature transition of poly(NIPAM) has been demonstrated already in previous studies.[13] We have also confirmed the complete reversibility of this transition. To study the dependence of parameters on the rate of the process we have measured $C_p(T)$ curves at four heating rates from 1.0 to 0.064 K/min. Table 1 shows that the middle point of the temperature transition (T_m), the calorimetric enthalpy of transition ΔH^{cal}, and the jump of the heat capacity at the transition $\Delta C_p(T_m)$ do not depend on the rate of heating, while ΔH^{eff} remarkably increases with a decrease of the heating rate. The value of ΔH^{eff} obtained by the extrapolation to very small heating rates (see Figure 2) is ~900 kcal/mol, while the value of ΔH^{cal} ~10^4 kcal/mol is more than 100 times larger. This means that the globule–coil transition of high molecular weight poly(NIPAM) is very far from an all-or-none one. We come to the conclusion that a high molecular weight poly(NIPAM) molecule consists of more than 100 coopertive units each with a molecular weight of about 6×10^4, which corresponds to about 500 monomers.

As one can see from Figure 2, the ΔH^{eff} value at the heating rate 0.064 K/min is near the value extrapolated to the zero rate. This means that the kinetic effects can be neglected at the heating rate 0.064 K/min. This fact confirms the observations of Meewes et al.[13] who employed a heating rate 10 times slower than that in our measurements but obtained the same transition temperature (34 °C) and observed no further changes when the heating rate was further lowered.

Discussion

This paper contains the first measurements of the cooperativity of the globule–coil transition in a synthetic homopolymer. Therefore it is interesting to compare the results with similar transitions in globular proteins. There are at least two types of cooperative conformational transitions in globular proteins. The first is protein denaturation, i.e. the loss of native tertiary 3D structure and activity. Sometimes in the course of this process a protein is transformed in a real unfolded (coil-like) state,[19] but in a number of cases a protein may be transformed in the "molten globule" state, which is compact but has no rigid tertiary structure, though probably preserves the general architecture of a native state (see ref 20 for a recent review). It has been shown[1,20] that the denaturation of the rigid 3D structure of a small protein molecule is an all-or-none process, i.e. a protein molecule loses its rigid structure as a whole when it transforms both to the unfolded and to the molten globule state. Moreover, recently, it was shown[21,22] that the loss of a compact (molten globule) state also involves a protein molecule as a whole. On the other hand, for large proteins both the *denaturation*,[2] i.e. the destruction of a rigid 3D structure, and *unfolding*, i.e. the destruction of a compact state (O. B. Ptitsyn and V. N. Uversky, unpublished results), are *not* all-or-none processes.

Large proteins as a rule consist of two or more quasi-independent structural lobes called "domains". The term "domain" has also been used[2] for a part of a large protein molecule which undergoes the transition as a whole (so-called "melting domain"). In a number of cases melting domains have been shown to coincide with independent lobes or "structural domains". However large proteins do not melt according to an all-or-none mechanism (not as a whole) even if they cannot be separated on two or more structural lobes (see e.g. refs 23 and 24).

In this paper we have shown that even a synthetic high molecular weight polymer does not undergo the globule–coil transition according to the all-or-none mechanism. This emphasizes that the absence of an all-or-none transition can be a general phenomenon for large macromolecules, including high molecular weight homopolymers. Using protein terminology, it means that even a homopolymer can consist of a number of melting domains.

It should be mentioned that we have shown the lack of all-or-none character of the coil–globule transition in a single high molecular weight polymer fraction. We expect that this character of transition will lead to the virtual independence of ΔH^{eff} of the molecular weight while ΔH^{cal} will be proportional to the molecular weight. The experimental work to check this point is now in progress.

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