Endothermic and exothermic components of an inverse temperature transition for hydrophobic association by TMDSC

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Abstract

Temperature modulated differential scanning calorimetry (TMDSC) has been used to study the phase separation process of three elastin-like model polymers; chemically synthesized poly(GVGVP) and genetically engineered $(GVGVP)_{251}$ and $(GVGIP)_{320}$. By these means, the characteristic endothermic peak found for these polymers in conventional calorimetry is revealed as being com-posed of two components, one endothermic due to loss of hydrophobic hydration and an oppositely signed exothermic component due to the physical association of chains (Vander Waals cohesive interactions) with the magnitude of the exothermic component being less than one-third that of the endothermic component. The magnitude of both components seems to depend mainly on the mean hydrophobicity of the monomer and not on the polymer molecular weight or dispersity.

1. Introduction

The inverse temperature transition ('ITT') has be-come the key issue in the development of new peptide-based polymers as molecular machines and materials. The understanding of the macroscopic properties of these materials in terms of the molecular processes taking place around the ITT has established a basis for their functional and rational design [1]. Moreover, this knowledge can be used to understand the relationship between folding and function in native proteins or the principles behind amphiphilic macromolecular assem-blies [1].

Poly(GVGVP) is the most renowned member of the elastin-like polymers (ELPs) (G≡glycine, V≡L-valine and P≡L-proline). This polymer can be considered a predominantly hydrophobic polypeptide, as deduced from its amino acid composition. Additionally, sys-tematic substitutions of the valines by an occasional amino acid residue with a different functional group or with a certain chemical modification thereof, have pro-duced different polymers that exhibit the capacity to perform the set of more than fifteen pairwise free energy transductions involving the intensive variables of me-chanical force, temperature, pressure, chemical poten-tial, electrochemical potential and electromagnetic radiation [2].

ELPs exhibit a reversible phase transitional behav-iour [2]. In aqueous solution and below a certain critical temperature (T_t), the free polymer chains remain dis-ordered, random coils in solution [3] that are fully hy-drated, mainly by hydrophobic hydration. This hydration is characterized by the existence of ordered clathrate-like water structures surrounding the apolar moieties of the polymer [1,4,5] with a structure somehow similar to that described for crystalline gas hydrates [5,6]. On the contrary, above T_t, the polymer

hydrophobically folds and assembles into nano and micro particles to form a phase separated state [7,8], in which the polymer chains adopt a dynamic, regular, non-random structure, called β-spiral, stabilized by intra-spiral inter-turn and inter-spiral hydrophobic contacts [2]. This is the product of the ITT. In this folded and associated state, the chain loses essentially all of the ordered water structures of hydrophobic hydration [1].

Hydrophobic hydration has been considered to play a relevant role in polymer functioning, folding and self-assembly for decades, particularly in proteins [5,9–12]. Nevertheless, the experimental approach to this type of hydration has been complex and not many contributing studies can be found in the literature since this effect is not predominant in natural proteins [13]. This drawback can be overcome the use of model ELPs [13], which, in addition, show two other valuable characteristics. For the model polymer studied here, pH does not significantly affect either their ITT or the stability of the folded state since their amino acids do not have any pH sensitive side chains [2]. Therefore, their ITT takes place just in simple water solutions and does not require the presence of a certain amount of salts or other compounds to get stable and functional conformations [2]. Second, their ITT is fully reversible [2]. Under static conditions, the folding state of the polymer is just a function of temperature, no matter the thermal history.

Differential scanning calorimetry (DSC) has proven to be an excellent tool to quantify many aspects of the ITT. In a typical DSC run, by which a cold water solution (below T_t) of an ELP is heated above T_t, one finds a characteristic endothermic peak. However, this thermal feature is actually the net result of two overlapping phenomena; i.e. destruction of ordered aqueous structures and chain folding and self-assembling. Since these phenomena are of such a different physical nature, a thermal method that provided an individual quantitative assessment of each component would be of great help in understanding the hydrophobic effect underlying the striking behaviour of this model family of advanced functional polymers.

Temperature modulated differential scanning calorimetry (TMDSC) provides an attractive opportunity. TMDSC is an improved DSC measurement that is able to separate thermally overlapping phenomena with different time dependences by using a heating program containing an alternating function of the temperature, such as a sinus, superimposed on the constant heating rate (v) [14–20]. In principle, TMDSC will provide a clear split of two overlapping phenomena when, under the particular dynamical conditions, one is reversible and the other is not.

In this Letter, we report the use of TMDSC to study the thermodynamic characteristics of the overlapping phenomena during the ITT exhibited by three different preparations of elastic model protein polymers.

2. Experimental

Chemically synthesised poly(VPGVG) (M_n = 96,155, $n = 1.18$) was obtained following the methods described earlier [21]. Detailed characterization of the final polymer can be found in [4 and 22]. $(GVGVP)_{251}$ and $(GVGIP)_{320}$ were prepared by recombinant DNA technology and expressed by *Escherichia coli* fermentation. Complete description of this procedure and essential characterizations of the gene product, $(GVGVP)_{251}$, have appeared elsewhere [23]. A report of the gene construction details for $(GVGIP)_{320}$ and extensive characterizations of the gene product are in preparation.

DSC and TMDSC experiments were performed on a Mettler Toledo 822^e with liquid-nitrogen cooler. Calibration of both temperature and enthalpy was made with a standard sample of indium. For DSC and TMDSC analysis, 125 mg ml^{-1} water solutions of the three polymers were prepared. In a typical DSC or TMDSC run, 20 μl of the solution were placed inside a standard 40 μl aluminium pan hermetically sealed. The same volume of water was placed in the reference pan. Both types of samples were pretreated 15 min at 5 °C inside the sample chamber just before the beginning of the experiment.

3. Results and discussion

Fig. 1 shows conventional DSC experiments for the three polymer solutions. The thermograms show the typical endotherm associated with the ITT. The thermal characteristics are as expected. $(GVGIP)_{320}$ showed the highest value of the transition enthalpy and the lowest T_t as a consequence of the increased hydrophobicity of the monomer GVGIP as compared to GVGVP. The (GVGVP) polymers showed the same transition en-

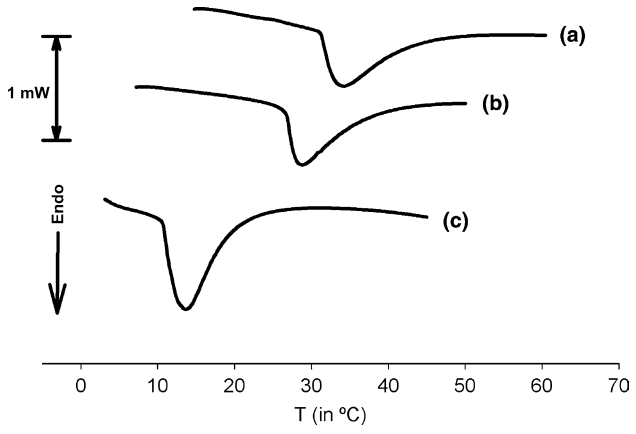

Fig. 1. Conventional DSC thermograms for 125 mg ml^{-1} water solutions of: (a) poly(GVGVP); (b) $(GVGVP)_{251}$; (c) $(GVGIP)_{320}$ (v = 10 °C min^{-1}).

thalpy as a result of their identical hydrophobicity but poly(VPGVG) showed a higher T_t value than $(GVGVP)_{251}$. This last effect is caused by the polydispersity of poly(GVGVP) and the influence of the low molecular weight fractions on T_t [7,24–26].

Hydrophobic hydration of this kind of polymer is characterized by the presence of ordered clathrate-like water structures surrounding the apolar moieties of the polymer [1]. Thus, on heating the polymer solution and surpassing T_t, these structures disappear. The destruction of the ordered hydrophobic hydration structures on heating must be considered as endothermic. On the contrary, once the polymer has lost its hydrophobic hydration, the resulting ordered folded state must be considered as an exothermic process. Thus, the endotherm found in the DSC runs must be the net result of the sum of both oppositely signed contributions.

Although both events take place concurrently, they have a very different nature. In particular, it is reasonable to consider that both phenomena occur with different kinetics. In effect, previous kinetic studies made on the same poly(VPGVG) used in this work showed than the process of phase separation is faster than the process of re-dissolution [27]. Therefore, both phenomena could be split by TMDSC if we are able to find a frequency for the periodic component low enough for the faster phenomenon to follow the oscillating temperature changes ('reversing') while high enough to impede this alternating behaviour of the slower one ('non-reversing').

Fig. 2a shows an example of the TMDSC thermogram found for $(GVGVP)_{251}$ while Fig. 2b shows the results of its analysis. As previously hypothesised, under those experimental conditions, the endothermic total curve ($\Delta H_{Tot} = -10.40$ J g^{-1}, $T_t = 27.72$ °C) is composed by a non-reversing endothermic component ($\Delta H_{non\text{-}rev} = -13.98$ J g^{-1}, $T_t = 27.63$ °C) and a reversing exotherm ($\Delta H_{rev} = 3.33$ J g^{-1}, $T_t = 27.30$ °C).

A detailed analysis has been carried out to study the dependence of the reversing and non-reversing components as a function of v, amplitude (A) and period (P). For the total contribution, the changes in v (0.5–1.5 °C/min), A (0.1–1 °C) and P (0.1–1.0 min) did not significantly affect the enthalpy and T_t values, which are similar to those obtained by DSC. Also the reversing and non-reversing components were not affected by changes in v and A (results not shown). However, P exhibits a strong influence on the enthalpy values of both components.

ΔH_{rev} has been plotted in Fig. 3 as a function of P for the three polymers. In all cases, at low frequencies (high P), the reversing component shows an endothermic peak with an enthalpy comparable to the one shown by the endothermic peak of the non-reversing component. Thus, at these high P, the chain folding and dehydration contributions were not well separated. However, as P decreases, ΔH_{rev} undergoes a substantial increase. At P about 0.8–1 min, the reversing component turns into a positive exothermic peak which reaches a maximum at $P = 0.5$–0.6 min (P_M). Parallelly, $\Delta H_{non\text{-}rev}$ suffers an equivalent decrease. Therefore, as P decreases, the reversing component is being enriched in the exothermic

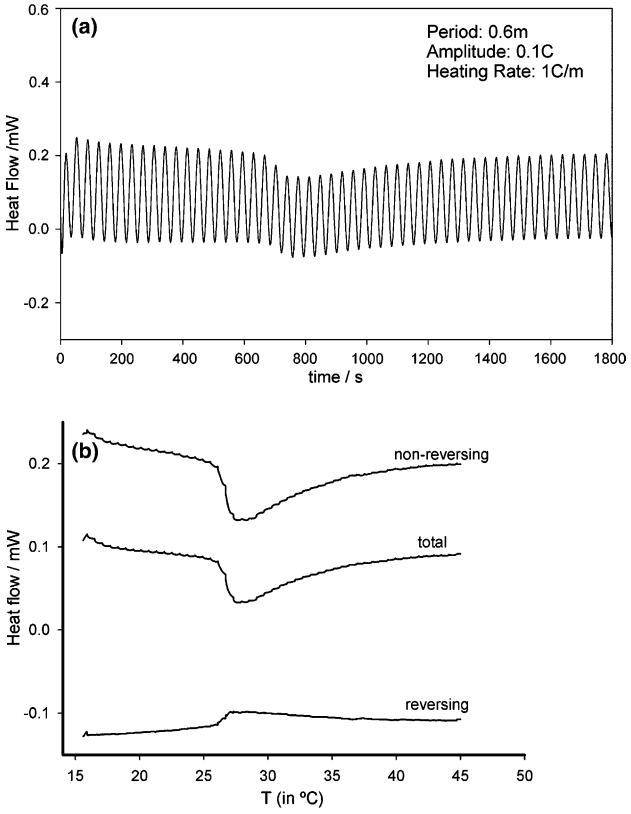

Fig. 2. (a) Heat flow vs. time in a TMDSC analysis of a 125 mg ml^{-1} water solution of $(GVGVP)_{251}$. (b) Reversing, non-reversing and total thermograms.

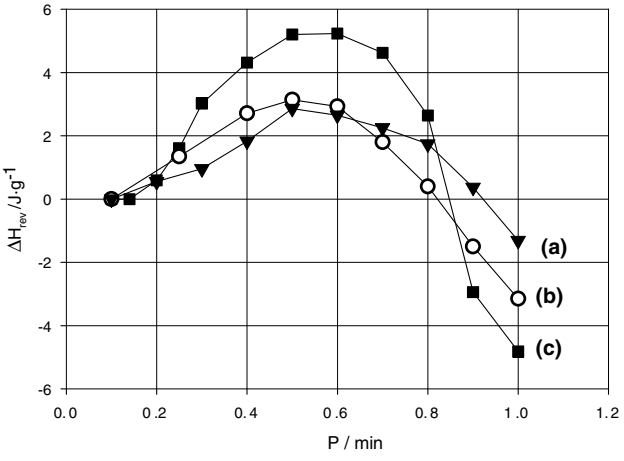

Fig. 3. ΔH_{rev} as a function of P for 125 mg ml^{-1} water solution of: (a) poly(GVGVP); (b) $(GVGVP)_{251}$; (c) $(GVGIP)_{320}$ ($v = 1$ °C min^{-1}, and $A = 0.1$ °C).

Table 1
Enthalpy values of the reversing, non-reversing and total components found at P_M

Polymer	ΔH_{rev} [J g^{-1}(kcal mol^{-1})]	$\Delta H_{non\text{-}rev}$ [J g^{-1}(kcal mol^{-1})]	ΔH_{tot} [J g^{-1}(kcal mol^{-1})]	P_M (min)
$(GVGIP)_{320}$	5.61 (177.4)	−22.82 (−672.6)	−17.21 (−507.2)	0.6
$(GVGVP)_{251}$	3.14 (77.14)	−11.34 (−278.6)	−7.50 (−184.2)	0.5
Poly(GVGVP)	2.96 (68.02)	−11.11 (−255.3)	−8.79 (−202.0)	0.5

component (chain folding), while the non-reversing is being enriched in the endothermic contribution (dehydration). The ΔH_{rev}, $\Delta H_{non\text{-}rev}$, ΔH_{Tot} values found at P_M can be seen in Table 1. Further decrease in P results in a progressive reduction in ΔH_{rev} to zero and an increase in $\Delta H_{non\text{-}rev}$ to the total enthalpy as a result of the complete overlap of both phenomena in the non-reversing component.

The maximum splitting was found at approximately the same P_M regardless of the polymer. Additionally, the comparison of the data found for $(GVGVP)_{251}$ and $(GVGIP)_{320}$ indicates that the reversing component at maximum is higher for $(GVGIP)_{320}$. Due to the higher hydrophobicity of I as compared to V, its chain folding has to show a higher exothermic ΔH_{rev} (see Table 1). Therefore, ΔH_{rev} values could then be used as a quantitative measurement of the amino-acid hydrophobicity. Additionally, the increased hydrophobicity of $(GVGIP)_{320}$ would also induce a higher extension of hydrophobic hydration, so its higher endothermic $\Delta H_{non\text{-}rev}$ is also reasonable.

There are no significant differences when comparing data from $(GVGVP)_{251}$ and poly(GVGVP) (see Table 1). Being the only difference between this two polymers their MW dispersity, their TMDSC results are practically the same, which would imply that the reversing and non-reversing TMDSC components depend mainly on the mean hydrophobicity of the monomer.

Typical literature ΔH values for the folding reaction of natural proteins are in the level of, −66.0 kcal mol^{-1} for ribonuclease or −12.4 kcal mol^{-1} for cytochrome c [28]. The ΔH_{Tot} values found for the ELPs used in this work are almost one order of magnitude higher than those values, which indicates the adequacy of these polymers as models of natural proteins. On the other hand, nonpolar gases are practically the only experimental system where hydrophobic hydration can be followed without interferences. In the alkane series, the enthalpy of solution in water is about −5 kcal mol^{-1} [29]. The values found here for $\Delta H_{non\text{-}rev}$ of ELPs are above this value but in the same order of magnitude. However, taking into account the huge differences in their molecular weights, the presence of less perfect structures of ordered water in the polymers compared to those taking place in nonpolar gases is plausible. Such heterogeneous structures with varying stabilities have been already suggested in [4].

4. Conclusions

TMDSC has demonstrated to be an effective method for separating overlapping phenomena present in the inverse temperature transition (ITT) of elastic protein-based polymers. By tuning the frequency of the periodic component, a maximum split can be achieved which shows an exothermic contribution arising from the Vander Waals contacts attending chain folding and assembly, and an endothermic contribution associated with loss of hydrophobic hydration, the former being about one fourth of the latter, in absolute values. The enthalpy values of both contributions seem to depend exclusively on the mean hydrophobicity of the monomer and not on other parameters such as molecular weight or polydispersity. To the best of our knowledge TMDSC is the only method currently available to separate both contributions. Accordingly, its utilization in future research to evaluate hydrophobicity of the full compliment of naturally occurring amino acids and relevant modifications thereof is clear and its relevance to hydrophobic folding of polymers and natural proteins is noteworthy.

Acknowledgements

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References

[1] D.W. Urry, J. Phys. Chem. B 101 (1997) 11007.
[2] D.W. Urry, Angew. Chem. Int. Ed. Engl. 32 (1993) 819.
[3] P.L. San Biagio, F. Madonia, T.L. Trapane, D.W. Urry, Chem. Phys. Lett. 145 (1988) 571.
[4] J.C. Rodríguez-Cabello, M. Alonso, T. Pérez, M.M. Herguedas, Biopolymers 54 (2000) 282.
[5] C. Tanford, The Hydrophobic Effect: Formation of Micelles and Biological Membranes, Wiley, New York, 1973.
[6] L. Pauling, E. Marsh, Proc. Nat. Acad. Sci. USA 38 (1952) 112.
[7] D.W. Urry, T.L. Trapane, K.U. Prasad, Biopolymers 24 (1985) 2345.
[8] M. Manno, A. Emanuele, V. Martorana, P.L. San Biagio, D. Bulone, M.B. Palma-Vittorelli, D.T. McPherson, J. Xu, T.M. Parker, D.W. Urry, Biopolymers 59 (2001) 51.

[9] W. Kauzmann, Adv. Protein Chem. (1959) 141.
[10] J.T. Edsall, H.A. McKenzie, Adv. Biophys. 16 (1983) 53.
[11] H.S. Frank, M.W. Evans, J. Chem. Phys. 13 (1945) 493.
[12] C. Chothla, J. Mol. Biol. 105 (1976) 1.
[13] D.W. Urry, S.Q. Peng, J. Xue, D.T. McPherson, J. Am. Chem. Soc. 119 (1997) 1161.
[14] M. Reading, Trends Polym. Sci. 1 (1993) 248.
[15] B. Wunderlich, R. Androsch, M. Pyda, Y.K. Kwon, Thermochim. Acta 348 (2000) 181.
[16] M. Reading, D. Elliott, V.L. Hill, J. Therm. Anal. 40 (1993) 949.
[17] G.S. GilI, S.R. Sauerbrunn, M. Reading, J. Therm. Anal. 40 (1993) 931.
[18] E. Verdonck, K. Schaap, L.C. Thomas, Int. J. Pharm. 192 (1999) 3.
[19] U. Jorimann, G. Widmann, R. Riesen, J. Therm. Anal. Calorim. 56 (1999) 639.
[20] J.D. Menczel, L. Judovist, J. Therm. Anal. 54 (1998) 419.
[21] D.W. Urry, J. Protein Chem. 7 (1988) 1.
[22] M. Alonso, D. Arranz, V. Reboto, J.C. Rodríguez-Cabello, Macromol. Chem. Phys. 202 (2001) 3027.
[23] D.T. McPherson, J. Xu, D.W. Urry, Protein Expr. Purif. 7 (1996) 51.
[24] D.W. Urry, D.T. McPherson, J. Xu, H. Daniell, C. Guda, D.C. Gowda, N. Jing, T.M. Parker, The Polymeric Materials Encyclopedia: Synthesis, Properties and Applications, CRC Press, Boca Raton, 1996, p. 7263.
[25] D.E. Meyer, A. Chilkoti, Biomacromolecules 3 (2002) 357.
[26] A. Girotti, J. Reguera, F.J. Arias, M. Alonso, A.M. Testera, J.C. Rodríguez-Cabello, Macromolecules (in press).
[27] J. Reguera, J.M. Lagarón, B. Calvo, V. Reboto, J.C. Rodríguez-Cabello, Macromolecules 36 (2003) 8470.
[28] P.L. Privalov, N.N. Khechinasvhvili, J. Mol. Biol. 86 (1974) 665.
[29] M.H. Abraham, A. Nasehzadeh, J. Chem. Soc., Faraday Trans. 77 (1981) 321.