Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Polymers 57 (2004) 293–297
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Effect of modified α, β, and γ-cyclodextrins on the thermo-responsive behavior of the elastin-like polymer, poly(VPGVG)

J. Reguera[a], M. Alonso[b], A.M. Testera[c], I.M. López[b], S. Martín[b], J.C. Rodríguez-Cabello[a,*]

[a]*Dpto. Física de la Materia Condensada, E.T.S.I.I., Universidad de Valladolid, Paseo del Cauce s/n, 47011 Valladolid, Spain*
[b]*Dpto. Química Analítica, E.U.P. Universidad de Valladolid, Francisco Mendizabal 1, 47014 Valladolid, Spain*
[c]*Dpto. Química Orgánica, E.T.S.I.I. Universidad de Valladolid, Paseo del Cauce s/n, 47011 Valladolid, Spain*

Received 3 March 2004; revised 19 April 2004; accepted 7 May 2004

Available online 9 June 2004

Abstract

The influence of modified α, β and γ-cyclodextrins (mCDs) on water solutions of the elastin-like thermo-responsive poly(VPGVG) has been studied in this work. The studied mCDs are hydroxypropyl-α-CD MS = 0.6, hydroxypropyl-β-CD MS = 0.6, hydroxypropyl-β-CD MS = 0.8, hydroxypropyl-β-CD MS = 1.0, hydroxypropyl-γ-CD MS = 0.6, hydroxyethyl-β-CD MS = 5.0 and methyl-β-CD MS = 1.8.

All mCDs were able to increase the temperature at which the inverse temperature transition of this polymer takes place (T_t). This shift was dose-dependent and was interpreted as the consequence of specific interactions between mCDs and the polymer chains. The ability showed by mCDs to cause T_t shifts was lower than that previously reported for unmodified CDs. However, due to the higher solubility of mCDs, some of the mCDs leaded to higher values of T_t than those found for the unmodified CDs at saturating concentration.

A continuous drop in the latent heat of the transition was also observed. This, in combination with the T_t shift to higher values, has been interpreted in terms of the reduction in the number of hydrophobic hydration ordered structures as the interaction between mCDs and the polymer chain takes place.

Keywords: Elastin-like polymers; Modified cyclodextrins; Inverse temperature transition; Smart polymers

1. Introduction

The model elastin-like polypentapeptide, poly(VPGVG) (G ≡ Glycine, V ≡ L-Valine and P ≡ L-Proline), is the head of this group of synthetic polypeptides (Urry, 1988, 1993; Urry et al., 1993a). This polymer is a thermo-responsive polymer since it shows reverse phase segregation when dissolved in water (Tamburro, Guantieri, Scopa, & Drabble, 1991; Urry, 1982, 1982, 1988b; 1993). Below a given temperature, the transition temperature (T_t), the chains in solution adopt a relatively extended and conformationally disordered disposition (Urry, 1993). As the temperature increases above T_t, the release of its hydrophobic hydration shell of the polymer chains allows inter and intra chain hydrophobic contacts which modify the assembly of the chain triggering a self-assembling process that finally lead to phase separation (Kokufuta, Ogane, Ichijo, Watanabe, & Hirasa, 1992; Miyamoto, Long, & Donkai, 1995). In this phase, the polymers chains show a regular folding in a helical structure called 'β-spiral'. This is formed by the concatenation of adjacent type II β-turns, one per pentamer. This spiral is further stabilized by hydrophobic interturn contacts (Kurkova et al., 2003; Urry, 1993). This transition is named *inverse temperature transition* ('ITT') (Urry, 1993). The existence of this ordered state in the segregated phase is a significant difference with other thermo-responsive polymers, so the conventional term LCST is not used in the context of elastin-like polymers (Lee, Macosko, & Urry, 2001; Urry, 1997).

Along with the change of order of the polymer chain in the ITT, there is also a change in the state of order of the water molecules surrounding the polymer. Below T_t, water forms ordered structures of hydrophobic hydration around the polymer chain stabilized by hydrogen bonding among themselves (Lee et al., 2001; Urry, 1993). These structures become unstable as the temperature increases and finally disorganize at T_t becoming bulk water. This disorganization

* Corresponding author. Tel.: +34-983-423680; fax: +34-983-423192.
E-mail address: cabello@eis.uva.es (J.C. Rodríguez-Cabello).

0144-8617/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2004.05.006

is accompanied by an isothermal heat absorption (ΔH_0) (Lee et al., 2001; Urry, 1993). Any modification in the composition of the polymer chain ('intrinsic') or addition of a substance ('extrinsic') that establishes a competence for water in terms of hydrophilic vs. hydrophobic hydration will alter the clathrate structure and, accordingly, will modify T_t and ΔH_0 (Urry, 1993). In general, T_t increases as the mean polarity increases and vice versa (Urry, 1993). This is the base of the ΔT_t mechanism by which some different smart polymers of this family have been obtained. These derivatives are able to isothermically respond to a great variety of external stimuli (Alonso, Reboto, Guiscardo, Martín, & Rodríguez-Cabello, 2000; Strzegowsky, Martinez, Godwa, Urry, & Tirrell, 1994; Urry, 1993, 1990; Urry, Hayes, Gowda, & Parker, 1991; Urry, Luan, & Cox, 1993b).

It is possible to intrinsically tune the T_t values by a proper design of the amino acid composition but the extrinsic control is, in principle, simpler because it can be achieved by adding adequate amounts of salts or organic products (Urry, 1993). This last effect has been already exploited in the use of elastin-like polymers for heavy-metal removing (Kostal & Mulchandani, 2001) and for the purification of elastin-like polymers for biomedical uses (Girotti et al., 2004; Panitch, Yamaoka, Fournier, Mason, & Tirrell, 1999). However, while extrinsic decreases in T_t can be achieved by a wide variety of additions (Urry, 1993), its extrinsic increase is more difficult to get. Initially, just a small set of substances, such as sodium dodecyl sulphate, guanidine hydrochloride, urea or triton X-100, have proven to induce very moderate increases in T_t (Urry, 1993). However, more recently, unmodified cyclodextrins (uDCs) showed a strong capacity to increase T_t, up to 20 °C for α-cyclodextrin (Alonso, Arranz, Reboto, & Rodríguez-Cabello, 2001). Furthermore, this increase was proven to be achieved by specific interactions between the uCDs and the polymer chains rather than the unspecific and inconvenient effect, in many cases, of the substances mentioned above. Additionally, the interaction with cyclodextrins (CDs) is the base of the recently described 'amplified ΔT_t mechanism', which has been first illustrated for an p-phenylazobenzene photoresponsive derivative of poly(VPGVG) (Rodríguez-Cabello, Alonso, Guiscardo, Reboto, & Girotti, 2002). In particular for this last one, UV–VIS spectroscopy has shown that the specific interaction between uCDs and the p-phenylazobenzene side chains of that elastin-like polymer was the formation of inclusion compounds (Rodríguez-Cabello et al., 2002).

However, the low solubility of uCDs limits their range of use and, thus, the final T_t shift achieved. Nevertheless, there is an alternative way to the use of uCDs that overcome the solubility problems of those. These are a set of modified CDs (mCDs) that can be produced and are commercially available. mCDs get a substantial increase of their solubility by substituting hydroxyl groups of the glucose units by hydroxypropyl, hydroxymethyl, methyl and hydroxyethyl groups. In order to test whether mCDs are able to promote higher T_t shifts than uCDs as a consequence of their higher solubility, the effect of a selected set of commercially available mCDs on the thermoresponsive behavior of poly(VPGVG) has been explored in this work.

2. Materials and methods

Hydroxypropyl-α-CD MS = 0.6 (Hpα0.6), hydroxypropyl-β-CD MS = 0.6 (Hpβ0.6), hydroxypropyl-β-CD MS = 0.8 (Hpβ0.8), hydroxypropyl-β-CD MS = 1.0 (Hpβ1.0), hydroxypropyl-γ-CD MS = 0.6 (Hpγ0.6), hydroxyethyl-β-CD MS = 5.0 (Heβ5.0) and methyl-β-CD MS = 1.8 (Mβ1.8) have been purchased from Sigma Chemicals. The average molar substitution degree (MS) expresses the number of added groups per glucose unit (Szejtli, 1992).

Poly(VPGVG) was synthesized in our laboratory as described by Lee and Fennema (1991) and Lovatt, Cooper, and Camilleri (1996). The studied polymer has an apparent average molecular weight, $M_n = 96,115$ and a polydispersity index, $n = 1.2$, as estimated by size exclusion HPLC. More details and data about synthesis and characterization of this polymer can be found elsewhere (Alonso et al., 2001; Rodríguez-Cabello, Alonso, Pérez, & Herguedas, 2000).

Differential Scanning Calorimetry (DSC) experiments were performed on a Mettler TA 4000 (DSC 30). Calibration of both temperature and enthalpy was made with a standard sample of indium. For DSC analysis, 125 mg/ml polymer solutions with the addition of variable amounts of the corresponding mCD (see text) were prepared. In a typical DSC run, 25 μl of the solution were placed inside a standard aluminum pan. The same volume of water was placed in the reference pan. Before the DSC run, the sample was pretreated at 5 °C for 30 min. That thermal adaptation was carried out inside the DSC instrument immediately before the DSC run. The heating rate was 10 °C/min and each thermogram was recorded from 5 to 90 °C. T_t is considered as the minimum in the endotherm and the peak enthalpy relates to the peak integral, in which a straight line is used as baseline (Alonso et al., 2001).

3. Results and discussion

The study of the inverse temperature transition and how it is affected by the presence of CDs can be conveniently carried out by calorimetric methods since they provide not only T_t values but also some other thermodynamic parameters of interest such as the transition enthalpy (ΔH_0) (Alonso et al., 2001; Rodríguez-Cabello et al., 2000; Urry, 1993;). The DSC thermogram of a water solution of poly(VPGVG) in presence or absence of CD is characterized by the presence of an endothermic peak on heating, corresponding to the transition from the unfolded and hydrated state to the folded and dehydrated one (Reguera et al., 2003; Urry, 1993)

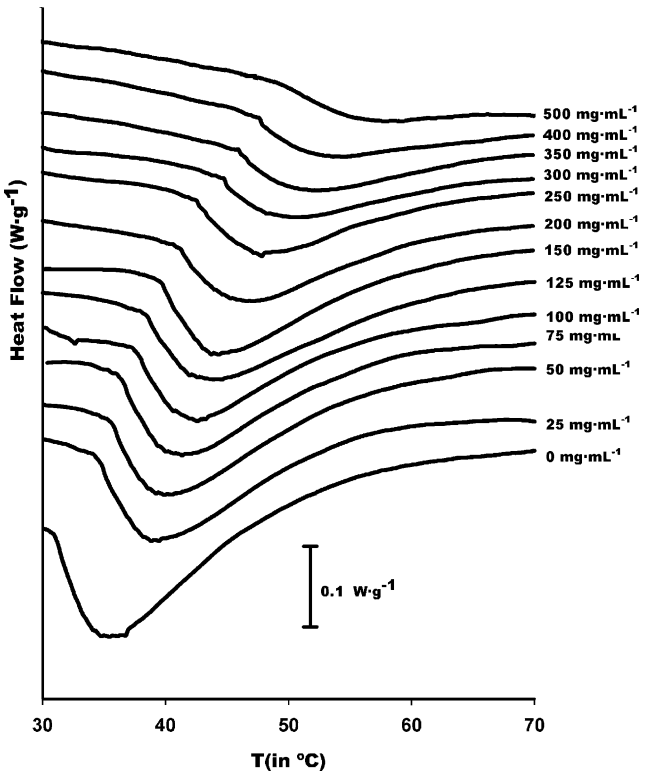

Fig. 1. DSC thermograms of 125 mg/ml water solutions of poly(VPGVG) in the presence of variying amounts of Hpα0.6. The concentrations of this last one are shown on the right.

where the enthalpy is composed of two components, one endothermic due to loss of hydrophobic hydration and an oppositely signed exothermic component due to the physical association of chains (Van der Waals cohesive interactions) with the magnitude of the exothermic component being less than one-third that of the endothermic component (Rodríguez-Cabello et al., 2004).

A typical dependence of the calorimetric features on mCD concentration has been plotted in Fig. 1 for Hpα0.6. From a qualitative point of view, two main facts can be pointed out from this figure. First, there is a continuous T_t shift to higher temperatures as the mCD concentration increases. Second, there is a decrease in ΔH_0, especially for high mCDs concentrations. All assayed mCDs caused, in more or less extent, the same effect; i.e. all of them promote a concentration-dependent T_t shift to higher temperatures and a decrease in ΔH_0.

The quantitative dependence of T_t on mCD concentration has been plotted in Fig. 2 for all mCDs. This quantitative analysis shows that, first, T_t shift does not show a clear linear trend. In general, it tends to show higher slope values for low mCD concentrations.

To discard unspecific actions of the mCD on this phase transition due to the saccharide nature of mCDs, DSC experiments were carried out with the substitution of mCDs by glucose, the main component of the mCDs, to a polymer solution similar to that used in the mCD experiments. The quantitative result of this work is also plotted in Fig. 2. The effect of glucose was clearly different to that of mCDs. This sugar caused a moderate decrease in the T_t value (-0.0135 °C/mg ml^{-1}). This limited and opposite behaviour of glucose points to a specific action of mCD on the thermal response of poly(VPGVG) in agreement to what was previously reported for the interaction of uCDs and poly(VPGVG) (Alonso et al., 2001), in which evidences on the formation of inclusion compounds between the cyclodextrins and the side chains of valine and proline are reported. The same specific effect was observed in a p-phenylazophenylalanine photoresponsive derivative of poly(VPGVG), in which the formation of inclusion compounds with these photochromic side chains could be easily observed by UV–VIS spectroscopy.

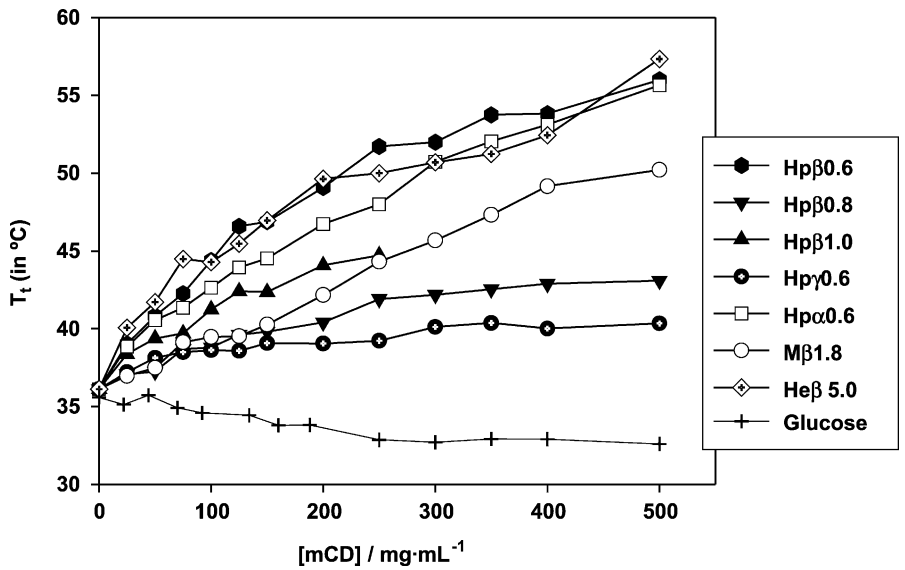

Fig. 2. Dependence of T_t as a function of [mCD] for the seven mCDs tested in this work and for the glucose. The labels inserted in the figure are to identify the corresponding mCDs and the glucose.

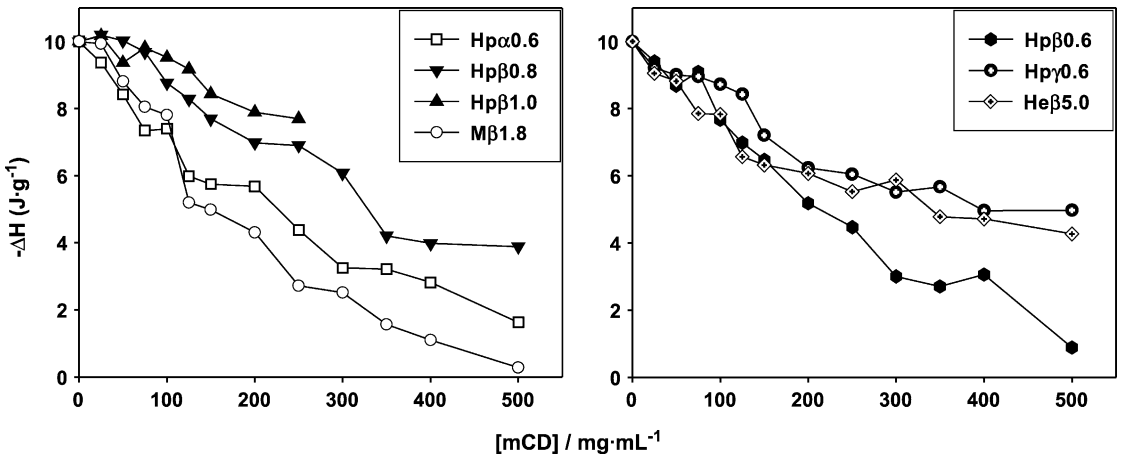

Fig. 3. Dependence of ΔH_0 as a function of [mCD] for the seven mCDs tested in this work. The figure has been divided in two plots only for clarity purposes. The labels inserted in the plots are to identify the corresponding mCDs.

A clear linear trend has been reported elsewhere for the dependence of T_t on uCD (Alonso et al., 2001). This linear trend was not reproduced here for mCDs (see Fig. 2). That different behavior likely relies on the low solubility of uCD. Due to that, the range of CD-polymer interaction was limited by this fact. Actually, for mCD concentrations in the range of those being saturating for uCDs (well below 200 mg/ml) the observed trend is linear, in practice, for all mCDs.

A second quantitative fact arising from the data of Fig. 2 is that the observed T_t shifts strongly depend on the particular mCD and not only on their concentration, indicating that the interaction between mCDs and the polymer strongly depends on CD shape and number and type of chemical modifications. This is evident, for example, analyzing the sequence Hpα0.6–Hpβ0.6–Hpγ0.6. In spite of their practically identical chemical composition and degree of substitution, they differing only in the size of the molecule, their effect on T_t were clearly very different. Hpβ0.6 showed one of the highest capacities to modify the T_t values among all mCDs tested while Hpγ0.6 showed the weakest influence. This fact is also in agreement with a specific interaction between the mCDs and the polymer. As an additional remark, unfortunately in these commercial mCDs, MS is defined as an average value. For each MS, there is not a defined molecular architecture but rather a complex mixture of mCDS. Therefore, the result of the interaction between these mCDs and the polymer is difficult to predict. This causes, for example, that for certain series, such as the Hpβ0.6, Hpβ0.8 Hpβ1.0, a monotonic trend in the variation of the calorimetric properties vs. MS has not been observed.

On the other hand, the power to shift the T_t values previously found for the three uCDs (Alonso et al., 2001) were clearly higher than the one found here for any mCD, as deduced from the slope of the plot of T_t vs. [uCD], even at low concentrations where the mCDs showed higher ability to shift this parameter. Apparently, the presence of these substituting groups (hydroxyethyl, hydroxyproyl or methyl) seems to hinder to some extension the interaction of the polymer chain and the mCDs as compared to the interaction with the unsubstituted ones. However, the clearly superior solubility of mCD compensates for most of them their weaker interaction so that the final T_t achieved (50.8 °C for Mβ1.8, 55.6 °C for Hpα0.6, 56.0 °C for Hpβ0.6 and 57.3 °C for Heβ5.0) was higher than the highest found for uCDs (50.6 °C for 170 mg ml^{-1} α-CD) (Alonso et al., 2001).

The analysis of ΔH_0 further supports the existence of an interaction that modifies the hydrophobic hydration structure of the unfolded state. As shown in Fig. 3, this parameter shows a continuous decrease as [mCD] increases. Since the endothermic peak of this transition is associated to the destruction of the ordered structures of hydrophobic hydration, a reduced ΔH_0 value would indicate a reduced amount of ordered water around the apolar moieties of the polymer.

Finally, although the observed T_t shift and the decrease in ΔH_0 are mainly caused by a specific interaction between the mCDs and the polymer chain, some additional effects such as, for example, the competition for water between mCD and the polymer in order to build their own hydration shell, cannot be discarded as an additional cause of T_t shift and ΔH_0 decrease, specially at the highest [mCD] tested, where water availability could be at compromise. Certainly, in a previous work (Rodríguez-Cabello et al., 2000), we have shown that in water deficiency conditions, the defective hydrophobic hydration of poly(VPGVG) tend to cause a moderate T_t shift to higher temperatures while accompanied by a small reduction in ΔH_0 values. In this sense, certain part of the T_t shift and the decrease in ΔH_0 found at high [mCD] could be caused by this phenomenon.

Acknowledgements

This work was supported by the 'Junta de Castilla y León' (Projects VA30/00B and VA002/02) and by

the MCYT (Projects MAT2000-1764-C02-02 and MAT2001-1853-C02-01).

References

Alonso, M., Arranz, D., Reboto, V., & Rodríguez-Cabello, J. C. (2001). Effect of α-, β- and γ-cyclodextrins on the inverse temperature transition of the bioelastic thermo-responsive polymer poly(VPGVG). *Macromolecules Chemistry Physics*, *202*, 3027–3034.

Alonso, M., Reboto, V., Guiscardo, L., Martín, A. S., & Rodríguez-Cabello, J. C. (2000). Spiropyran derivative of an elastin-like bioelastic polymer: photoresponsive molecular machine to convert sunlight into mechanical work. *Macromolecules*, *33*, 9480–9482.

Girotti, A., Reguera, J., Rodríguez-Cabello, J. C., Arias, F. J., Alonso, M., & Testera, A. M. (2004). Design and bioproduction of a recombinant multi(bio)functional elastin-like protein polymer containing cell adhesion sequences for tissue engineering purposes. *Journal of Materials Science Materials M*, *15*, 479–484.

Kokufuta, E., Ogane, O., Ichijo, I., Watanabe, S., & Hirasa, O. (1992). Poly(vinyl methyl-ether) gel for construction of a thermosensitive immobilized enzyme-system exhibiting controllable reaction initiation and termination. *Journal of the Chemical Society, Chemical Communications*, *99*, 416–418.

Kostal, J., Mulchandani, A., & Chen, W. (2001). Tunable biopolymers for heavy metal removal. *Macromolecules*, *34*, 2257–2261.

Kurkova, D., Kriz, J., Schmidt, P., Dybal, J., Rodriguez-Cabello, J. C., & Alonso, M. (2003). Structure and dynamics of two elastin-like polypentapeptides studied by NMR spectroscopy. *Biomacromolecules*, *4*, 589–601.

Lee, J., Macosko, C. W., & Urry, D. W. (2001). Phase transition and elasticity of protein-based hydrogels. *Journal of Biomaterials Science Polymer Edition*, *12*, 229–241.

Lee, M. J., & Fennema, O. R. (1991). Ability of cyclodextrins to inhibit aggregation of beta-casein. *Journal of Agricultural and Food Chemistry*, *39*, 17–21.

Lovatt, M., Cooper, A., & Camilleri, P. (1996). Energetics of cyclodextrin-induced dissociation of insulin. *European Biophysics Journal*, *24*, 354–357.

Miyamoto, T., Long, M., & Donkai, N. (1995). Preparation of new types of temperature-responsive cellulose derivates. *Macromolecular Symposium*, *99*, 141.

Panitch, A., Yamaoka, T., Fournier, M. J., Mason, T. L., & Tirrell, D. A. (1999). Design and biosynthesis of elastin-like artificial extracellular-matrix proteins containing periodically spaced fibronectin CS5 domains. *Macromolecules*, *32*, 1701–1703.

Reguera, J., Lagaron, J. M., Alonso, M., Reboto, V., Calvo, B., & Rodriguez-Cabello, J. C. (2003). Thermal behavior and kinetic analysis of the chain unfolding and refolding and of the concomitant nonpolar solvation and desolvation of two elastin-like polymers. *Macromolecules*, *36*, 8470–8476.

Rodríguez-Cabello, J. C., Alonso, M., Guiscardo, L., Reboto, V., & Girotti, A. (2002). Amplified photoresponse of a p-phenylazobenzene derivate of elastin-like polymer by α-cyclodextrin: the amplified Δt_t mechanism. *Advanced Materials*, *14*, 1151–1154.

Rodríguez-Cabello, J. C., Alonso, M., Pérez, T., & Herguedas, M. M. (2000). Differential scanning calorimetry study of the hydrophobic hydration of the elastin-based polypentapeptide. Poly(VPGVG), from deficiency to excess of water. *Biopolymers*, *54*, 282–288.

Rodríguez-Cabello, J. C., Reguera, J., Alonso, M., Parker, T. M., McPherson, D. T., & Urry, D. W. (2004). Endothermic and exothermic components of an inverse temperature transition for hydrophobic association by TMDSC. *Chemical Physics Letters*, *388*, 127–131.

Strzegowsky, L. A., Martinez, M. B., Godwa, D. C., Urry, D. W., & Tirrell, D. A. (1994). Photomodulation of the inverse temperature transition of a modified elastin poly(pentapeptide). *Journal of the American Chemical Society*, *116*, 813–814.

Szejtli, J. (1992). The properties and potential uses of cyclodextrin derivatives. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, *14*, 25–36.

Tamburro, A. M., Guantieri, V., Scopa, A., & Drabble, J. M. (1991). Polypeptide models of elastin: CD and NMR studies on synthetic poly(X-Gly-Gly). *Chirality*, *3*, 318–323.

Urry, D. W. (1982). Characterization of soluble peptides of elastin by physical techniques. *Methods Enzymology*, *82*, 673–717.

Urry, D. W. (1984). Protein elasticity based on conformations of sequential polypeptides: the biological elastic fiber. *Journal of Protein Chemistry*, *3*, 403–436.

Urry, D. W. (1988). 'Bioelastics' break new ground in the development of biomaterials. *Reserach and Development*, *8*, 1–8.

Urry, D. W. (1988b). Entropic elastic processes in protein mechanisms. I. Elastic structure due to an inverse temperature transition and elasticity due to internal chain dynamics. *Journal of Protein Chemistry*, *7*, 1–34.

Urry, D. W. (1990). Preprogrammed drug delivery systems using chemical triggers for drug release by mechanochemical coupling. *Polymer Material Science and Engineering*, *63*, 329–336.

Urry, D. W. (1993). Molecular machines: How motion and other functions of living organism can result from reversible chemical changes. *Angewandte Chemie International Edition England*, *32*, 819–841.

Urry, D. W. (1997). Physical-chemistry of biological free-energy transduction as demonstrated by elastic protein-based polymers. *Journal of Physical Chemistry B*, *101*, 11007–11028.

Urry, D. W., Hayes, L. C., Gowda, D. C., & Parker, T. M. (1991). Pressure effect on inverse temperature transitions: biological implications. *Chemical Physics Letters*, *182*, 101–106.

Urry, D. W., Luan, C. H., & Cox, B. A. (1993b). In G. Lenaz (Ed.), (*Vol. 4*). *Proteins I: structure, hydrophobicity, elasticity and energy conversion in treatise on bioelectrochemistry: biomacromolecules, Bologna, Italy*.

Urry, D. W., Nicol, A., Gowda, D. C., Hoban, L. D., McKee, A., Williams, T., Olsen, D. B., & Cox, B. A. (1993a). In C. G. Gebelein (Ed.), *Biotechnological polymers: Medical, pharmaceutical and industrial applications*. Atlanta, GA: Technomic Publishing.