Thermochimica Acta, 227 (1993) 167–183
Elsevier Science Publishers B.V., Amsterdam

A calorimetric study of cooperative phase equilibria in microbial polysaccharides [1]

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(Received 8 April 1993; accepted 20 April 1993)

Abstract

Some calorimetric data of the order–disorder conformational transitions of natural polysaccharides from microorganisms are presented in the more general thermodynamic framework of the cooperative helix–coil transition of linear biopolymers. A new home-made data acquisition and processing procedure is presented and discussed. The phase transition is analysed for a neutral capsular polysaccharide from *Rhizobium trifolii* TA1 and for two ionic exopolysaccharides, xanthan from *Xanthomonas campestris* and succinoglycan from a strain of *Rhizobia*. Structural features, such as the linear charge density, are discussed as determining factors in the thermal stability of the helical ionic polysaccharides.

INTRODUCTION

Among all known polysaccharides, only those secreted by microorganisms can be obtained in a reproducible way from cultures of carefully selected strains and in principle they are not subject to random changes in chemical structure. From the thermodynamic point of view, this regularity of primary structure involves the possibility that the chains take on ordered helical conformations of either a single- or multiple-strand type, both in the solid state and in solution, with important outcomes for their capability to form supramolecular structures up to the gel phase with peculiar rheological properties [1–3]. The persistence of regular stable conformations is, however, limited to segments, which are always much shorter than the actual chain length. The knowledge of the energetics of this variety of

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[1] Presented at the 14th National Conference on Calorimetry and Thermal Analysis, Udine, Italy, 13–17 December 1992.

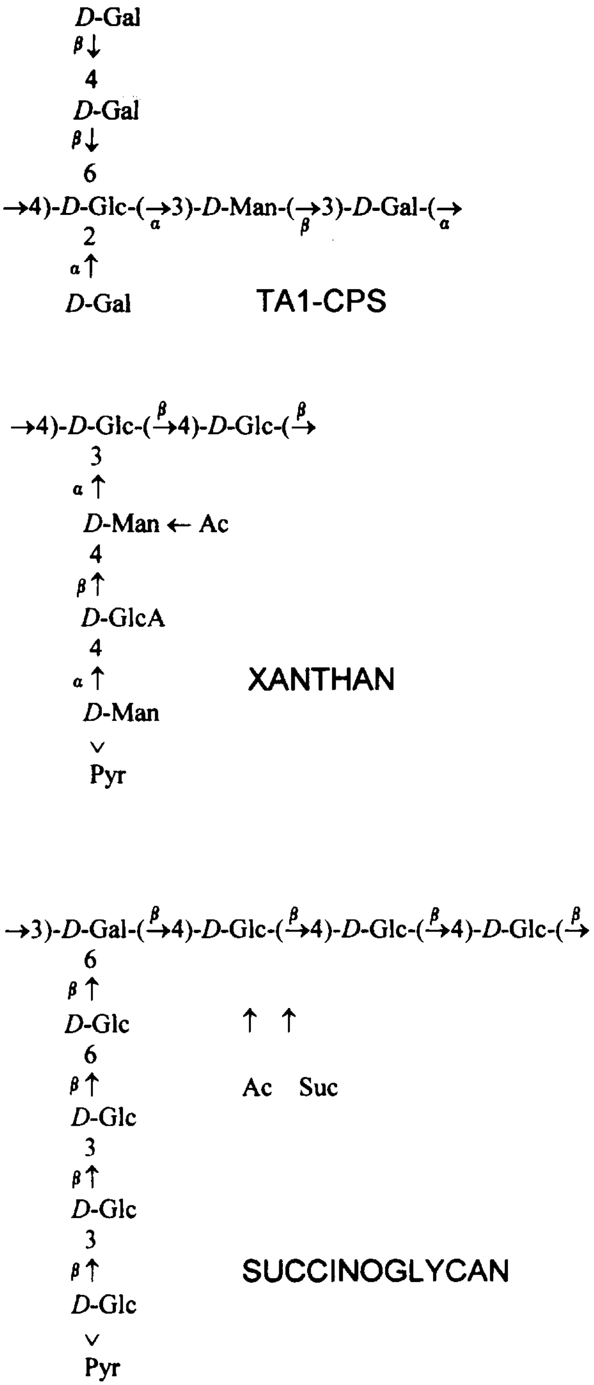

Fig. 1. Structure of the repeating unit of capsular polysaccharide TA1-CPS from *Rhizobium trifolii*, and of xanthan and succinoglycan.

conformations up to the three-dimensional molecular shapes and of the interaction between the polymer and other solution components is essential in order to understand, control and upgrade the macromolecular properties in industrial applications. Although some notable examples exist in the literature, only a few microbial polysaccharides have been studied calorimetrically, including a derivation of the cooperativity.

Here we present a preliminary analysis of calorimetric results on the thermal stability and phase transitions of some industrial important polysaccharides derived from microorganisms. The examples reported (Fig. 1) include the capsular polysaccharide from *Rhizobium trifolii* TA1(TA1-CPS), some samples of xanthan (from *Xanthomonas campestris*) and a succinoglycan obtained from *Rhizobium meliloti* strain YE2(S1).

THE THERMODYNAMICS OF THE HELIX–COIL TRANSITION IN LINEAR BIOPOLYMERS

Thermodynamic arguments [4] suggest that the partially disordered state is an essential prerequisite for the stability of polymeric systems in solution. Under these circumstances, realistic chain pictures of polysaccharides will not necessarily be generated from a single minimum energy state found in the internal conformational energy calculations. Some of these conformational energy surfaces [5] suggest a more disordered solution conformation than the chain structures proposed in order to fit the helical regularity deduced from X-ray fibre diffraction studies. Thermal fluctuations are sufficient to generate delocalized disorder, unless diffuse interactions, cooperative in nature, ensure a statistical long-range order. This conformational variability of linear biopolymers is also recognizable by the difficulty in crystallization and in the typical phenomenon of polymorphism.

There is, however, empirical evidence for the presence of an ordered phase which may correspond to a conformation with minimum energy and which is further stabilized by a number of external variables (salt, pH, etc.). Given the small difference in energy (per monomer unit of the macromolecule), the transition process occurs at temperatures higher than ambient only if a portion of the whole macromolecule can be thermodynamically considered a single domain [6]. The hypothesis is made that the disordering is effectively a "cooperative" process between two distinct states, thermodynamically defined and in equilibrium with each other at the transition temperature.

The stability of ordered structures is a function of chain-length m, with a critical value of the chain-length above which the helix is interrupted, a concept introduced in the Zimm–Bragg theory [7] through the cooperativity parameter σ. This parameter essentially describes the excess free energy of formation of an isolated helical conformation with respect to that for the same process occurring as a neighbour of a helical sequence, for which the free energy change associated is described by the parameter s. The statistical-mechanical matrix model developed for the helix–coil transition of linear polypeptides has already been generalized [8] to include other parallel phenomena, such as, for example, the zippering of ordered chains in double or triple helices, and has also been used to treat the binding of small iodine molecules into the amylose core which effectively induce the ordering conformational transition [9]. Under this theoretical approach, the partition function is written as $\mathbf{Z} = \mathbf{P}\mathbf{U}^m\mathbf{Q}$, where the statistical weight matrix $\mathbf{U}$ is properly indexed for every nearest-neighbour interaction on the polymer, each element in the matrix giving the relative probability (statistical weight) for finding site i $(1 < i < m)$ in a particular state (helical or coil). Proper differentiation of the partition function with respect to the statistical weights give the thermodynamically averaged quantities of interest, such as the average number of helical segments $\langle N_h \rangle$ and the average length of the helical segments, $\langle L_h \rangle$

$$\langle N_h \rangle = \mathrm{d} \ln Z / \mathrm{d} \ln s$$

and

$$\langle L_h \rangle = \mathrm{d} \ln Z / \mathrm{d} \ln \sigma \qquad (1)$$

The σ parameter is related to the sharpness of the change in any property measured as a function of a variable inducing helix–coil transition. The prediction of the theoretical treatment is that the cooperativity of the transition depends on the chain length m and on the parameter σ, whilst the average transition temperature depends on m and mainly on the value of s.

From the thermodynamic point of view, this premise is not complete, however, because the transition of an ionic biopolymer from an "ordered structure" with a given conformation to an extended coil conformation is usually associated with a net variation of the ionic charge density along the chain. The polyelectrolytic counterion condensation (CC) theory [10] has provided a tool for describing several properties of solutions of ionic polymers in terms of the structural parameter ξ, unequivocally defined as $e^2/DkTb$, where e is the value of the elementary charge, D is the dielectric constant of the medium, k is the Boltzmann constant, T is the temperature (Kelvin), and b is the distance between the projections of the fixed charges of the polyelectrolyte on its contour axis. Application of this theory is successful with many experimental data, and, in particular, an extensive correlation has been made between the theoretical predictions and the isothermal calorimetric data on the processes of protonation, dilution and mixing with ions [11].

In addition to the counter-ion condensation phenomena, other physical responses may also occur simultaneously, which may mask the central statement of the CC theory that the onset of the critical value ξ (for univalent ions $\xi > 1$) constitutes a thermodynamic instability which must be compensated by counterion condensation. In fact, chain elongation and/or chain disaggregation occurs or changes with variation in charge density, and the energy instability effectively becomes a function of the thermodynamic state of the polyelectrolytic chain.

For thermally induced, conformational transitions between states i and f of a polyelectrolyte, characterized by a set of ξ_i and ξ_f (i.e. b_i and b_f) values, the CC theory predicts a simple relationship [10] between the values of the melting temperatures (T_M, the temperature of transition midpoint) and the logarithm of the ionic strength I

$$\frac{d(\log I)}{d(T_M^{-1})} = -\frac{\Delta_M H}{9.575 F(\xi)} \tag{2}$$

where $\Delta_M H$ is the value of the enthalpy of transition (in J per mole of charged groups) determined calorimetrically. This linearity implies that the enthalpy change is essentially due to non-ionic contributions and largely independent of I. The function $F(\xi)$ depends on the charge density of both the final state (subscript f) and the initial state (subscript i), within the common condition that $\xi_f < \xi_i$, i.e. the final state is characterized by a smaller value of the charge density. The value of $F(\xi)$ in three cases is

$$\text{if both } \xi_i \text{ and } \xi_f < 1, \qquad F(\xi) = (\xi_f - \xi_i) \tag{3a}$$

$$\text{if both } \xi_i \text{ and } \xi_f > 1, \qquad F(\xi) = (\xi_f^{-1} - \xi_i^{-1}) \tag{3b}$$

$$\text{if } \xi_i > 1 \text{ and } \xi_f < 1, \qquad F(\xi) = (2 - \xi_f - \xi_i^{-1}) \tag{3c}$$

These relations have been successfully applied to the transition processes of DNA [10], polynucleotides [12], and of some ionic polysaccharides (carrageenans [13], xanthan [14, 15], etc.).

Therefore, under the assumption usually made, the heat of transition evaluated by DSC experiments ($\Delta_M H$) appears in the equation reported above for the conformational transition of helical ionic biopolymers but differs from that evaluated using the van't Hoff isochore (ΔH_{vH}) for the apparent equilibrium constant. This discrepancy is a direct consequence of, and theoretically related to, the existence of molecular blocks which undergo a cooperative phase transition with a change of enthalpy which is larger than the unitary change, i.e. per residue, by a factor of $\sigma^{-1/2} \approx \langle L_h \rangle$, already defined as the length of the cooperative unit. Calorimetric measurements directly provide the value of $\langle L_h \rangle$ as the ratio of the apparent van't Hoff and the calorimetric heat of transition, $\Delta H_{vH}/\Delta_M H$, provided that the polymer chain length is much larger than the length of the cooperative unit.

EXPERIMENTAL

Samples

The sample of capsular polysaccharide from *Rhizobium trifolii*, strain TA1 (TA1-CPS) and the samples of xanthan (PS556 and PS646da, with different pyruvate contents) were kindly provided by Dr. L.P.T.M. Zevenhuizen and by Dr. I.W. Sutherland, respectively. The sample of succinoglycan was extracted from a fermentation broth kindly provided by the Environmental Polytech Consortium (CO.E.PO.), using the procedures described by Zevenhuizen and Faleschini [16]. Chemical analyses and physico-chemical properties of the batch samples have already been reported [16, 17]. The molecular weights, evaluated by means of different methods, are 3×10^6 for TA1-CPS, and 1×10^6 for xanthan, and 0.18×10^6 for succinoglycan.

The polysaccharides were obtained as freeze-dried solids, containing approximately 13.5%–14.5% of water (w/w). Solutions were prepared by dissolving the solid samples in water or in the proper concentration of salt solution to obtain the final desired polymer concentration. When necessary, solutions were heated before filling the calorimetric cells. Particular care was taken in balancing the two cells in order to get a proper zero displacement in the signal.

Methods

The system used was a MicroDSC Setaram microcalorimeter. A DASM-4 microcalorimeter was also used for TA1-CPS. The two cells were filled with the sample and with the reference solutions, particular care being given to the weight balance of the two cells, in order to have both the heating and cooling profiles within the assigned range. In each measurement a cyclic heating and cooling programme was followed with at least four heating scans, in order to check for the reversibility. Reversibility was not obtained with xanthan samples. With sample PS556, the area of the melting endotherm decreased dramatically after the first run, while the area of the endotherm increased by annealing the sample PS646da for four hours at a temperature of about 10 degrees below the melting temperature.

A schematic representation of the instrumental setup and of the sequence of steps for the processing of the data is given in Fig. 2. The output signal of the microcalorimeter was connected to an IBM PC through a Burr–Brown A/D interface, yielding parallel acquisition of the digital and analogue data on the chart recorder [18]. The capability of the digital acquisition interface and the real time software, coupled with the architecture of the IBM microprocessor, made it possible to select a frequency of up to 1000 data points per second with a real time control of

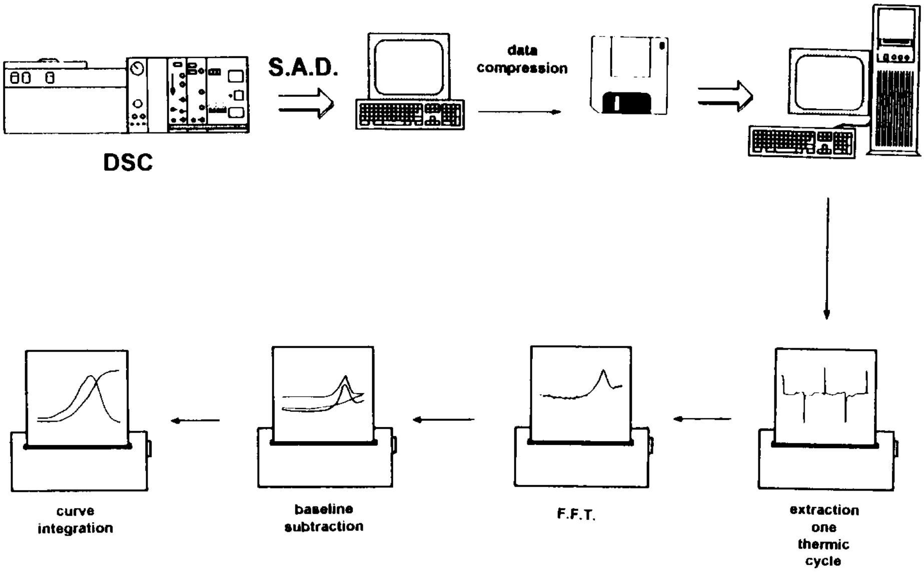

Fig. 2. Schematic assembly of the Setaram MicroDSC with the computing options.

the amplification of up to 100 times. The data acquisiton software (SAD) collects all data points in a single file of sequential data, which are normalized and formatted in such a way that the file can then be transferred to a larger computer for the analysis. This solution was preferred in order to dedicate the IBM-PC to the acquisition function with the highest controlling speed and to develop more sophisticated software for the data processing.

Data processing

Mathematical elaboration (BRAINME) of the data file was carried out with different options. The routine analysis involves first the selection of the correct portion of the raw data for a heating or cooling cycle, followed by a data-rejection option, in order to have discordant data rejected if they deviate from the mean by more than four times the average deviation on a suitable data sample basis. Typically, a whole curve was scanned at $0.2\,\mathrm{K\,min^{-1}}$ with a frequency of 1 or 10 Hz giving either 300 or 3000 data points per degree, with a total of 21 000 or 210 000 points for the interval from 20 to 90°C. Each of these segments was automatically analysed for correcting possible local splattering of the data.

Alternatively, the signal was subjected to a fast-fourier-transform (FFT) analysis to improve the signal-to-noise ratio, due to electrical fluctuations and statistical variation in environmental conditions; then subtraction of the proper baseline was performed. After filtering the noise interference, very reproducible heat capacity data were obtained and it was possible to ascertain any minimal distortion of the thermal profiles during repeated heating–cooling cycles.

The thermodynamic model

The helix–coil transition theory formulates the microscopic process of transition in a generalized way. However, some of the polysaccharides are believed to occur in a multiple helical assembly. At this preliminary stage of analysis, no unbiased model can be assumed to correlate the experimental quantities with the molecular parameters. To avoid these complications, only the overall cooperativity was studied and the comparison is made within the same class of polymers. In particular, xanthan has been proved [19] to be affected by both the thermal and salt concentration "history", which may largely modify the percentage of double and single helical conformations in what is believed to be the "native" form of the biopolymer.

RESULTS

The capsular polysaccharide from Rhizobium trifolii *TA1*

The most dramatic solution property exhibited by TA1-CPS is, by far, its ability to form aqueous thermo-reversible gels in a wide range of polymer concentrations (down to $0.1\ \mathrm{g\,l^{-1}}$). In particular, due to the non-ionic character of the polysaccharidic chain, gels can be formed in the absence of ionic cosolutes, and these show a remarkable gel strength.

Calorimetric experiments have been carried out with three different high-sensitivity DSC instruments (in different laboratories) under similar conditions [20]. The polysaccharide was repeatedly heated and cooled, and the thermal curves were not only almost completely reproducible, but also the response of the different instruments were in satisfactory agreement. The DSC results (Fig. 3) show a very sharp transition at around 47°C, although the transition starts at lower temperatures. The transition is reversible, sharp but asymmetrical, and for the polymer concentration of $4\ \mathrm{g\,l^{-1}}$ it gives a calorimetric heat of transition $\Delta_M H$ of $22.2 \pm 0.4\ \mathrm{J\,g^{-1}}$ and a van't Hoff enthalpy of about $1255\ \mathrm{kJ\,mol^{-1}}$ (of repeat units, RU), based on the chemical structure of the repeat unit characterized by a trisaccharide in the chain backbone which possesses two branches on the same glucosidic residue. According to the procedure outlined in ref. 6, these data make it possible to determine the length of the cooperative unit, which was estimated as defined by about 57 RU, or in other units by a mass of 57000.

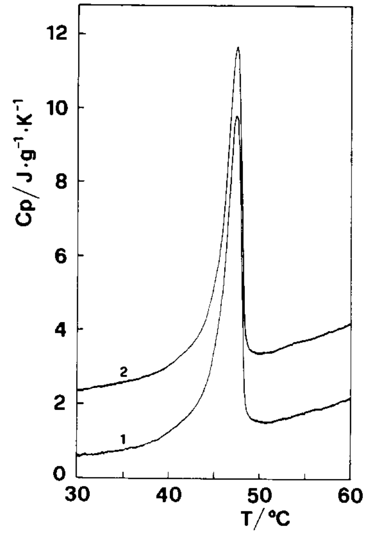

Fig. 3. DSC thermograms (first (1) and second (2) heating scans) of TA1-CPS in water obtained with a DASM-4 microcalorimeter. Experimental conditions: $C_p = 4\ \mathrm{g\ l^{-1}}$; scan rate, 0.1277 K $\mathrm{min^{-1}}$.

The concentration dependence can be inferred from the comparison of the different data shown in Table 1 and reveals the energetics of the chain–chain interaction which provides the formation of the gel phase.

Although the quality of the diffraction pattern of the TA1-CPS did not enable a good resolution of its structure, a pseudo-double-helix (2-fold

TABLE 1

Calorimetric data for TA1-CPS and succinoglycan

C_s [a]/M	C_p [b]/g $\mathrm{l^{-1}}$	T_M/°C	$\Delta_M H$ [c]/J $\mathrm{g^{-1}}$	$\Delta H_{vH}/\Delta_M H$ [d]
TA1-CPS				
0	4.0	47.2	22.2	57
0	2.1	46.8	17.0	≈60
0	1.0	46.0	15.3	65
0	0 [e]	45.2	12.6	–
Succinoglycan				
0	2	58.2	2.3	≈330
0	4	59.8	8.2	72
0	8	63.2	10.0	55
0.01	4	65.1	9.2	8
0.10	4	74.4	11.3	10
1.0	4	80.2	12.0	11

[a] Concentration of added salt (NaCl). [b] Concentration of polymer. [c] Enthalpy of transition, measured by calorimetry. [d] Cooperative length (ref. 6), $\langle L_n \rangle$ given in repeat units. [e] Extrapolated at zero polymer concentration.

symmetry) has been recently claimed, with a chain repeat axis of 1.01 nm per repeating unit [21]. This work does not substantially change the previous value of 0.98 nm proposed by the same authors, but with a different structure. From these data, a length of about 60 nm for the cooperative segments can be assumed, if this is the only cooperative structure which stabilizes the ordered helical conformation in the gel structure.

A number of other experimental observations [22, 23], in particular on the thermal and rheological behaviour of TA1-CPS in the presence of co-solutes (urea, salt, or sucrose), suggest that at least three different levels of structure may be involved in the process of aqueous gel formation [24, 25]. The first level has been referred to as local chain conformational ordering and corresponds to the process of enthalpy 12.6 J g^{-1}. The second level has been thought to involve "intermolecular ordering between conformationally ordered segments" and may correspond to an enthalpy change of about 2.6 J g^{-1}. This second level of structure has been shown to resist shear and such denaturants as urea. The last level of structure provides for the three-dimensional gel network and is labile under moderate shear and in concentrated urea solution: it involves supramolecular aggregation. Evidence showing a complex aggregation in the development of the gel structure has also been accumulated by experimental work carried out independently by Gidley et al. [23]. In particular, both the typical hysteresis in the melting–setting thermal cycles and the temperature dependence of the rigidity (storage) modulus in water and in aqueous urea solution, support the presence of an intermediate step for the formation of aggregate structures.

A possible hypothesis for such gelling behaviour is that the stereoregular non-ionic side chains of CPS, which eventually determine the formation of the gel, maintain a helical conformation in water (very likely the same as has been found for CPS fibres by means of X-ray diffraction [21]). The gel network is then stabilized by an array of energetically favourable overlaps between the side chains of different molecules [25]. In the absence of a clear picture of the molecular model for the gel junctions, it is not possible to speculate further on the meaning of the cooperative length found from calorimetric experiments. This number seems to be related to the size of the thermodynamic domains of the polymer undergoing the transition, without claiming that it is associated to a pair or to more than two chains.

Xanthan

The polysaccharide secreted by *Xanthomonas campestris,* xanthan, has a $(1\rightarrow4)$-β-D-glucan backbone with a trisaccharide side-chain attached on alternate glucosyl residues (Fig. 1). Acetyl and pyruvate groups are present with at most one group of each per repeating unit. The proportion of these groups, which strongly affect the properties of the solution and their thermal stability [26–28], can be changed by mild chemical modification or by changing strain and culture conditions. Most of the studies have been carried out either with native or with chemically modified samples. Only recently, several native samples of xanthan were produced with different amounts of non-sugar substituents. The non-linear changes in several of their physico-chemical properties (when heated in the range 0–100°C) were explained with a thermally induced cooperative transition. Two different models have been proposed for describing the secondary (and tertiary) structure of xanthan, on the basis of different experiments in solution. The fibre diffraction results are compatible with either of the two proposals, the double-chain model being more widely accepted [29]. Not even the "disordered" state has been verified as a "real" random coil, although flexibility and disorder are enhanced with respect to the native conformation [30].

The thermodynamic analyis of the conformational transition (see eqn. (2)) is based on the assumption that the enthalpy change $\Delta_M H$ is essentially due to non-ionic contributions and largely independent of the ionic strength [10]. This hypothesis is verified by using the recent data of Shatwell et al. [27] on native samples with different amounts of acyl substituents (Fig. 4). The different samples show only slight changes in the value of the slope and, furthermore, one of the xanthan samples (PS556) has a bimodal phase transition, each step of which is characterized by a similar value of the non-ionic enthalpy change (properly normalized per mole of charges).

The transition behaviour of xanthan has also been monitored using many different methods [14], e.g., viscometry, chirooptical and light scattering measurements, calorimetry, etc. However, the authors have not been able to reach a conclusive understanding of the molecular structure and the molecular basis for the conformational transition due to conflicting

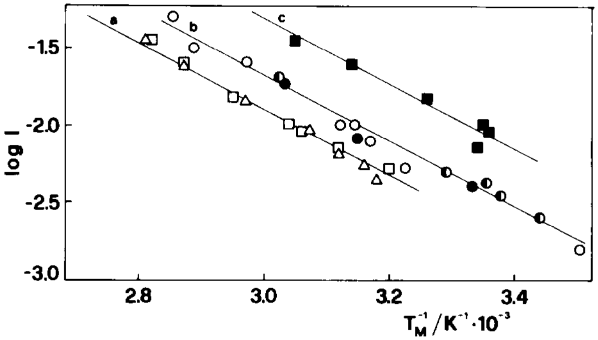

Fig. 4. "Phase diagram" of the Na^+ salt form of xanthan samples with different acetyl and pyruvyl contents (see text and ref. 27). Curve a: △, PS646da; curves a and c, □, ■, PS556. Curve b, data from ref. 14 on xanthan commercial samples; symbols refer to different methods of monitoring the thermal transition: optical methods, ○, and ◐; viscometry, ●.

reports, which often originate from the sample history [19]. Here, we limit our attention to the ionic strength dependence of the conformational transition. Literature data [14] of the temperature of transition obtained by optical measurements (optical rotation, circular dichroism and birefringence), together with the viscometric results of our laboratory, are reported in Fig. 4 for commercial xanthan samples under different conditions of ionic strength. The slope of the linear plot is −2090 ± 70 K which could be used to evaluate the r.h.s. term of eqn. (2), provided that either the enthalpy change or the change of charge density are known for the disordering process.

The calorimetric results on our xanthan samples indicate a substantial dependence of each individual sample on the thermal history. This is shown by the raw thermograms (Fig. 5) obtained with PS64da, characterized by a moderate amount of pyruvyl and acetyl substitution (the molar substitution is 0.37 and 0.28, respectively). When the sample is subjected to repeated heating and cooling cycles, its transition moves to a higher temperature and the peak becomes sharper. The phenomenon probably has the same origin as the "annealing" process for semi-crystalline polymers and demonstrates the metastable conditions of the formation of the weak gel system.

Opposite results have been obtained with the sample PS556, with a high pyruvyl and moderate acetyl content (0.62 and 0.34, respectively). With

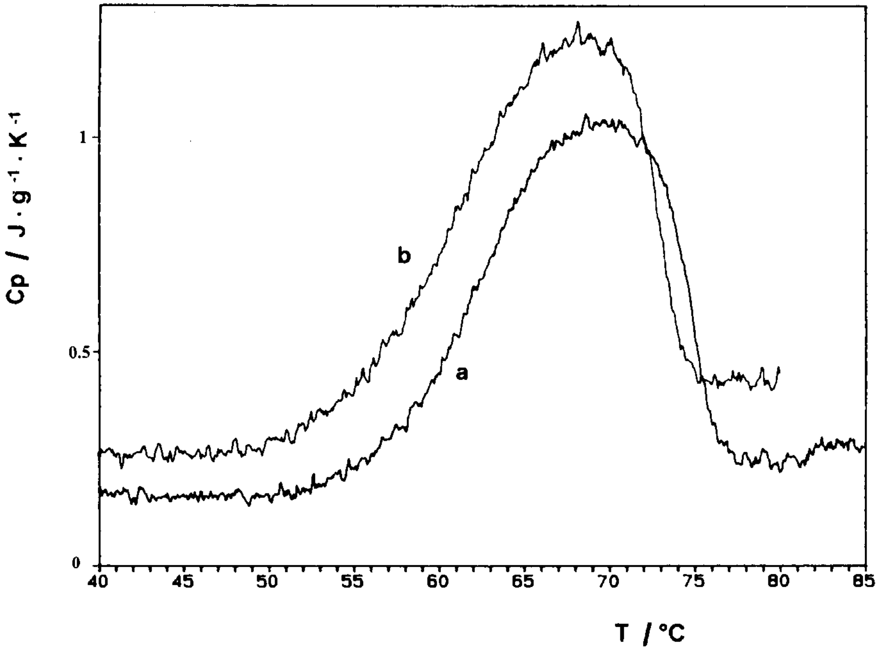

Fig. 5. DSC thermograms (a, heating; b, cooling) of PS646da xanthan sample in 0.1 M NaCl. Experimental conditions: $C_p = 10\ \mathrm{g\ l^{-1}}$; heating rate, 0.2 K min^{-1}.

this sample the thermograms deteriorate after the first heating cycle, which questions the primordial "native" stage of the sample. Shatwell et al. [27] found a two-step transition behaviour for this xanthan sample and could not find a reasonable explanation. From these observations it can be suggested that a high heterogeneity occurs in the substituent distribution, giving some block patterns which create a damping effect for the renaturation. In fact, one of the two-step transitions presents a thermodynamic stability comparable with the other samples, while the other step is much more unstable. Although no reliable data are yet available on the direct calorimetric evaluation of the energetics of the two transitions, the preliminary conclusions inferred from the data of the phase diagram alone are consistent with the interpretation of an energetically similar unfolding process for the block sequences in the PS556 sample.

For the value of the cooperativity, values of $\langle L_h \rangle$ ranging from 40 to 120 have been reported for different samples under different experimental conditions [15], but without any mention of the thermal treatment given to the samples, or of the possible reversibility observed. The results obtained with our PS646da sample give a value of $\langle L_h \rangle$ ranging between 40 and 50, reaching the higher value asymptotically after prolonged exposure to thermal cycles.

Succinoglycan

Several experimental observations [31–33] consistently show the occurrence of a temperature-induced order-to-disorder transition for succinoglycan aqueous solutions. The mid-point transition temperature T_M depends on the salt concentration, ranging from 66 to 72°C. Upon cooling, a considerable hysteresis occurs in the typical sigmoidal trend of the optical activity as a function of temperature [31, 33] in salt-free solutions. The phenomenon depends on the ionic strength, and is absent in salt concentrations above 0.1 M [32]. In all cases, the original optical activity and molar ellipticity values are recovered after a single thermal cycle and a suitable cooling time of the solutions.

As for other biopolyelectrolytes, the melting temperature T_M increases on increasing the ionic strength. Most of the experimental points of the literature for the dependence of $-\log I$ against $1/T_M$ fall, with reasonable approximation, on a straight line over the whole range of ionic strength investigated (Fig. 6). The slope of the plot ($-14.75\ 10^3$ K) gives the l.h.t. of eqn. (2), while the r.h.t. contains the enthalpy of transition and the change in the charge density ξ with the transition. An enthalpy of transition of 11 kJ per charge of polymer has been reported for a commercial sample from *Pseudomonas* in aqueous 0.1 M salt solution. Very surprisingly, a dependence of the enthalpy of transition between 9 and 12 J g^{-1}, i.e. between 7.4 and 9.8 kJ per charge of polymer, was found in the present investigation on

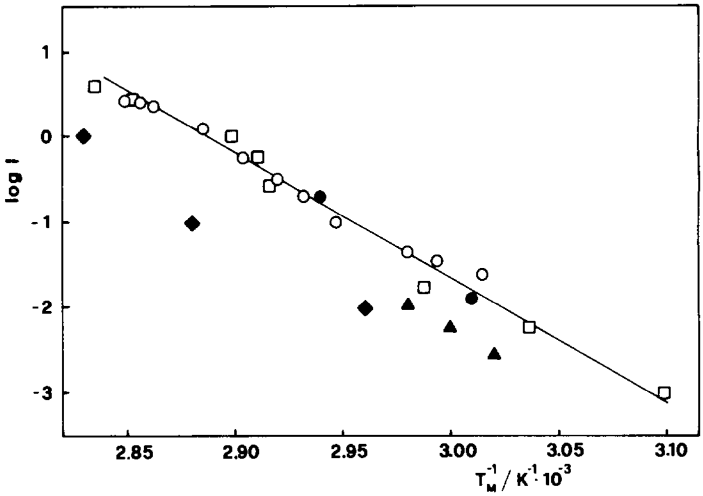

Fig. 6. "Phase diagram" of the Na^+ salt form of succinoglycan. Data from refs. 30–32 (○) and 34 (□) refer to thermal transitions monitored by optical activity, viscometry, osmotic coefficients and DSC (●). Data on samples from *Rhizobium meliloti* (◆, ▲) were recorded by DSC.

the succinoglycan from *Rhizobium meliloti* by increasing the ionic strength from 0.01 M to 1 M (see Table 1). The change in ξ, i.e. the value of $F(\xi) = \xi_f - \xi_i$, which can be calculated by using any of these values for the $\Delta_M H$ is quite small, about 0.05. This is an extremely low change in charge density. Furthermore, although not surprisingly, the enthalpy of transition for the polymer in water was also found to depend strongly on the concentration (Fig. 7), an indication of the dilution-induced disordering process exhibited by other biopolymers.

Data from light scattering and viscosity experiments [31–33] suggest that the native succinoglycan from *Pseudomonas* in aqueous solution adopts a rather stiff single-helix conformation; the estimated Khun length is 285 nm, as compared with 255 nm for xanthan. A ratio of 1.3 between the experimental and the expected single-strand mass per unit length has been calculated from light scattering data, suggesting that some chain association may be present.

Succinoglycan samples from different fermentation conditions seem to exhibit different rheological behaviour. A remarkable and irreversible decrease in viscosity of high molecular weight succinoglycan from *Pseudomonas* has been interpreted in terms of cleavage of the polymer backbone or disruption of aggregates upon heating. The tendency to form aggregates may also be derived from the value of the mass per unit length as obtained by light scattering experiments. No such irreversible behaviour was found for the low molecular weight sample of succinoglycan used in the present investigation. The role played by the side chain on the aggregation

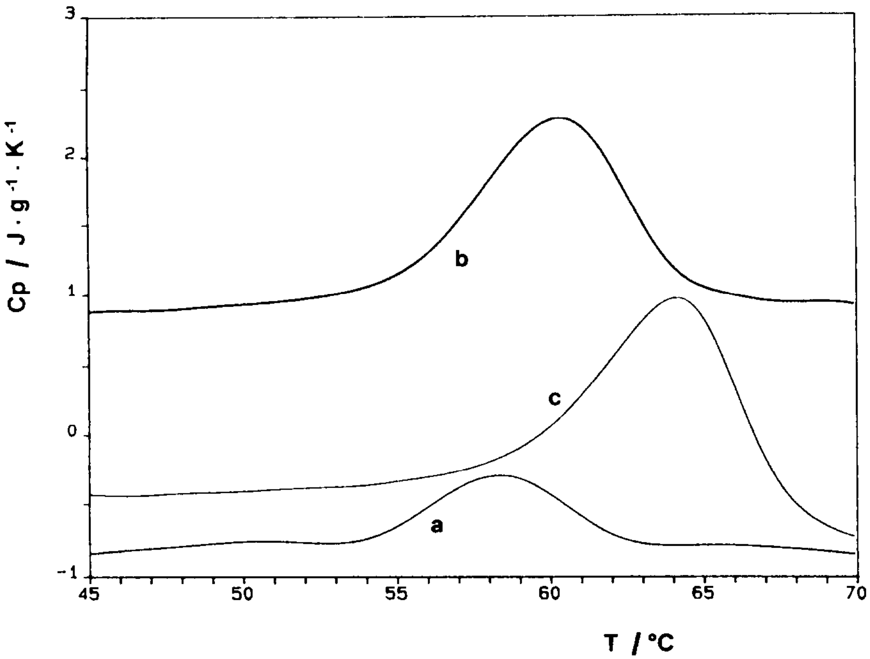

Fig. 7. DSC thermograms of succinoglycan in water: a, $C_p = 2\,\mathrm{g\,l^{-1}}$, b, $4\,\mathrm{g\,l^{-1}}$; c, $8\,\mathrm{g\,l^{-1}}$.

process results from the solution properties of succinoglycan after the removal of pyruvate and/or succinate groups. The removal of succinate groups does not prevent the formation of aggregates, as can be deduced from the irreversible decrease in intrinsic viscosity; on the contrary, the pyruvate-free samples show a complete reversibility in the intrinsic viscosity after the thermal cycle. Therefore, the influence of the charged substituents on the conformational stability follows the trend shown by xanthan [26], indicating a common mechanism for the role played by the charged substituents on the conformational stability of polysaccharides in aqueous solution.

CONCLUSIONS

All the evidence reported here suggest the occurrence of a true phase transition in polysaccharides under given circumstances. Direct calorimetric measurements of both the heat of transition and of its partial derivative have been shown to be invaluable for quantifying the cooperative character of the polymeric native chain.

Independent research into the rheological properties of concentrated solutions of these polysaccharides has produced data which are interpreted on the basis of the polymer conformation and chain rigidity [3]. In fact, worm-like polysaccharides with low flexibility show a remarkable deviation from the behaviour described for flexible, statistically disordered chains. The viscoelastic spectrum of the former resembles that of a typical gel system, indicating that non-transient supramolecular structures also occur, although these weak gel systems flow on increasing shear. This behaviour, typical of so-called weak gels, has been claimed to reflect the occurrence in the polymer network of weak non-covalent intermolecular forces. Persistence of these weak interactions over an extended length of the chain is conceivable within a fraction of regular structures. It is the authors' intention to claim that the energetics and the cooperativity of the phase transition are just another aspect of the macroscopic solution behaviour of the gelling-ordered polysaccharides. Molecular description of these structures has not yet been achieved and other experimental data are necessary to substantiate these hypotheses.

ACKNOWLEDGMENTS

This research activities leading to this work were partially developed under a contract of the National Program of Research on Advanced Innovative Materials by the Environmental Polytech Consortium (CO.E.PO.) N. 139026-1209/302 from the Italian Ministry of University and Scientific Technological Research and with the financial support of the Progetto Finalizzato Chimica Fine II, through CNR (Roma).

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