Biophysical Chemistry 11 (1980) 387–395
© North-Holland Publishing Company

CONFORMATIONAL TRANSITIONS OF THE HYDROPHOBIC POLYACIDS

Shintaro SUGAI and Nobumichi OHNO *
Department of Polymer Science, Faculty of Science, Hokkaido University, Sapporo, Hokkaido 060, Japan

The conformational transitions of the alternating maleic acid copolymers with styrene $(\text{MA-St})_n$ and α-methyl styrene $(\text{MA-MSt})_n$ in aqueous solutions were studied by means of various methods. The following results were obtained: 1) The conformational transitions of $(\text{MA-St})_n$ from the compact to extended coil form are observed in various salt solutions, as in aqueous NaCl, and the compact form is stabilized by Rb^+ and Cs^+, but destabilized by Li^+. The coions, Br^-, I^-, ClO_4^- and SCN^- affect scarcely the stability of the compact form. 2) The temperature coefficient of viscosity $d \ln [\eta]/dT$ of $(\text{MA-St})_n$ in 0.09 M NaCl was positive for the compact form, but negative for the coil form, and it reflects the transition. 3) The difference between specific heats for the compact and coil forms of $(\text{MA-St})_n$ in 0.03 M NaCl is determined to be about 15% of the corresponding heat of transfer of benzene to aqueous medium. 4) A remarkable dilution of the bound monomeric acridine orange to the compact form $(\text{MA-St})_n$ is observed and the dimerization free energy of the dye in the compact form is about −2.1 kcal/mole at 25°C. 5) Potentiometric, dilatometric and viscometric titrations of $(\text{MA-MSt})_n$ in aqueous NaCl at 25°C show a similar conformational transition to that of $(\text{MA-St})_n$. Also, the difference in the molar extinction coefficient at 261 nm indicates the transition. The compact form of $(\text{MA-MSt})_n$ is more unstable than that of $(\text{MA-St})_n$. From the results, the compact conformations and the transition mechanism of both the polyacids were discussed in comparison with the results for the maleic acid copolymers with *n*-alkyl vinyl ethers.

1. Introduction

Various synthetic polyelectrolytes are known to exist in a somewhat compact conformation at small degrees of ionization α. Poly(methacrylic acid) (PMA) in the compact form has been extensively investigated [1–5], and the form is considered to be stabilized by short range interaction between methyl groups. Long range electrostatic interactions become predominant at high α, and the compact form is converted into an extended coil form. Some copolymers of maleic acid and *n*-alkyl vinyl ethers have been found to undergo similar transitions upon ionization of the primary carboxyl groups [6–10]. Both the van der Waals and hydrophobic interactions seem to be responsible for stabilization of the compact form.

A similar pH-induced conformational transition of an alternating copolymer of maleic acid and styrene $(\text{MA-St})_n$ has been recently found [11–14], and a thermodynamic analysis of the transition in aqueous NaCl solution was carried out by us with data for the fractionated samples by means of various methods including potentiometric, viscometric, dilatometric, optical and calorimetric ones [11,12]. The compact form at small degrees of ionization α_1 of the primary carboxyl groups was shown to be not so compact as in the globular protein, and considered to be stabilized by hydrophobic interaction between phenyl groups buried in the copolymer and the residue exposed after the transition. The standard enthalpy change, ΔH_t^0, estimated by calorimetry at 25°C was positive corresponding to the positive transfer heat of benzene to aqueous medium.

In analyzing pH and optical titration results of $(\text{MA-St})_n$, the conformational transition was assumed, as in the case of PMA, to be attributed to the transition between two segmental forms (the *g*-form, in which the segment is buried in the hydrophobic domain, and the *c*-form, in which it is exposed). The midpoint of transition calculated from the pH-titration data coincided with that from the optical ones. The initiation parameter σ of the g-form calculated by use of Zimm–Rice theory was found to be similar to those for the maleic acid copolymers of *n*-alkyl vinyl ethers obtained by Dubin and Strauss [8].

---

* Permanent address: Department of Industrial Chemistry, Akita Technical College, Akita, Japan.

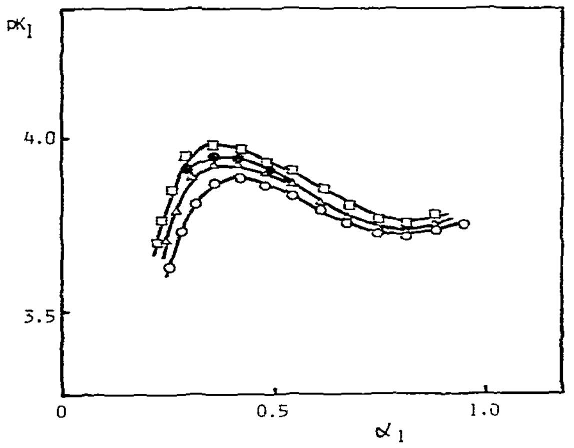

Fig. 1. The modified pH-titration curves of $(MA\text{-}St)_n$ of MW = 613 000 in aqueous solutions of various sodium salts at I = 0.09 and 25°C: NaCl (○), NaBr (△), NaSCN (●) and $NaClO_4$ (□). (To avoid overlapping, the NaI data are not given.) The polymer concentration c_p is 0.0091 g/ml.

On the other hand, kinetic studies on the transition from the compact form were performed by a pH-jump method [15–16]. The kinetic trace was analyzed by applying the general kinetic theory of cooperative transition, and a linear Ising model was found to be applicable even for the compact form to interpret the dependence of the mean relaxation times on α_1. Applicability of such a model may show the importance of the short range interaction between the hydrophobic residues for stabilization of the compact form of $(MA\text{-}St)_n$. Also, the σ-value was calculated from the kinetic data and found to agree with the values from the equilibrium methods.

The aim of our study is to obtain information about the compact conformations of the hydrophobic polyacids and to clarify the transition mechanism. In this paper are reported the conformational transition studies of the alternating maleic acid copolymers of styrene and α-methyl styrene $(MA\text{-}MSt)_n$ in aqueous salt solutions by means of various methods. Moreover, interactions of the hydrophobic dye, acridine orange (AO), with both the copolymers are described.

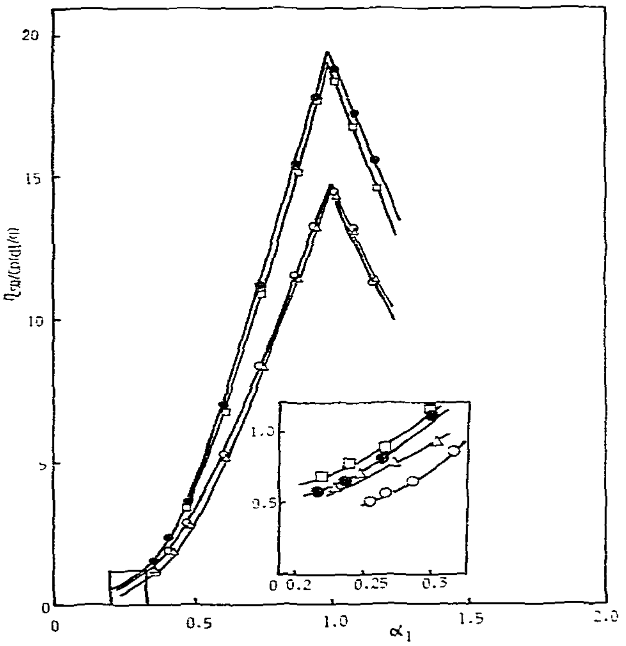

Fig. 2. The reduced viscosity of $(MA\text{-}St)_n$ in aqueous salt solution at I = 0.09 and 25°C. The symbols, MW and c_p the same as in fig. 1.

2. Materials and methods

The fractionated $(MA\text{-}St)_n$ was obtained by the method described previously [11]. The alternating copolymer of maleic anhydride and α-methyl styrene $(MAn\text{-}MSt)_n$ was made from a mixture of maleic anhydride and α-methyl styrene in decalin, α,α′-azobisisobutyronitrile being used as an initiator [17]. Fractionation was achieved by slow addition of poor solvents. The approximate molecular weight (MW) was estimated from a relation between the intrinsic viscosity [η] and MW of $(MAn\text{-}St)_n$ [18]. $(MAn\text{-}MSt)_n$ was hydrolyzed in water at about 80°C. The elementary analysis of $(MAn\text{-}MSt)_n$ and the pH-titration of $(MA\text{-}MSt)_n$ showed that the obtained sample is a 1 : 1 copolymer of maleic acid and α-methyl styrene.

AO (Merck) was recrystalized in a mixture of ethanol and water. Its concentration was determined from the molar extinction coefficient at 492 nm [19]. Other chemicals used were of special grade (Nakarai Chem., Kyoto).

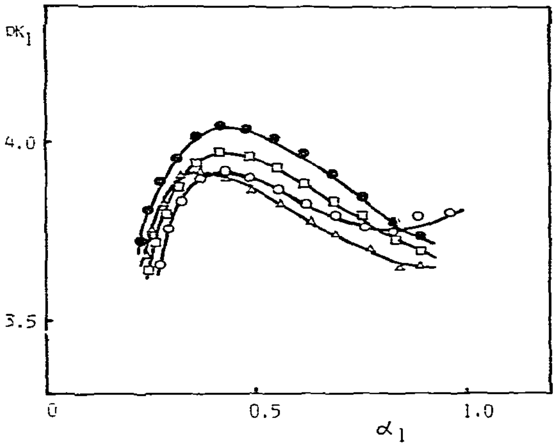

Fig. 3. The modified pH-titration curves of $(MA\text{-}St)_n$ in aqueous solutions of various chlorides at $I = 0.09$ and 25°C: NaCl (○), LiCl (△), CsCl (●) and RbCl (□). MW and c_p the same as in fig. 1.

pH, viscosity, volume change, calorimetric and optical measurements were described previously [11–12, 15–16,20].

3. Results

3.1. Conformational transition of $(MA\text{-}St)_n$

3.1.1. Effects of counterions and coions on the transition

In fig. 1, the pH-titration data of $(MA\text{-}St)_n$ in aqueous solutions of various sodium salts are shown. The coions, Br^-, I^-, ClO_4^- and SCN^-, are found to affect scarcely the modified titration diagram. In fig. 2, effects of the coions on the reduced viscosity of $(MA\text{-}St)_n$ in the compact form are shown. Although the effects are not so remarkable, the reduced viscosity of the compact form copolymer depends slightly on the coion species. Especially, the water structure breaking ions affect the viscosity for the compact form. One of the causes for stabilization of the compact form of $(MA\text{-}St)_n$ seems to be the water structure around the molecule.

The counterion affects the stabilization of the compact form, as shown in figs. 3 and 4, where Cs^+ and Rb^+ are shown to be bound more tightly than Na^+ to the compact form copolymer and to stabilize the form. However, Li^+ destabilizes the compact form and expands the molecular dimension. Although the side chains of *n*-alkyl vinyl ether copolymers are reported to bind Li^+, which stabilizes the compact form [10], interactions of Li^+ with charged carboxyl groups, phenyl groups and water structure may be taken into consideration in the present case.

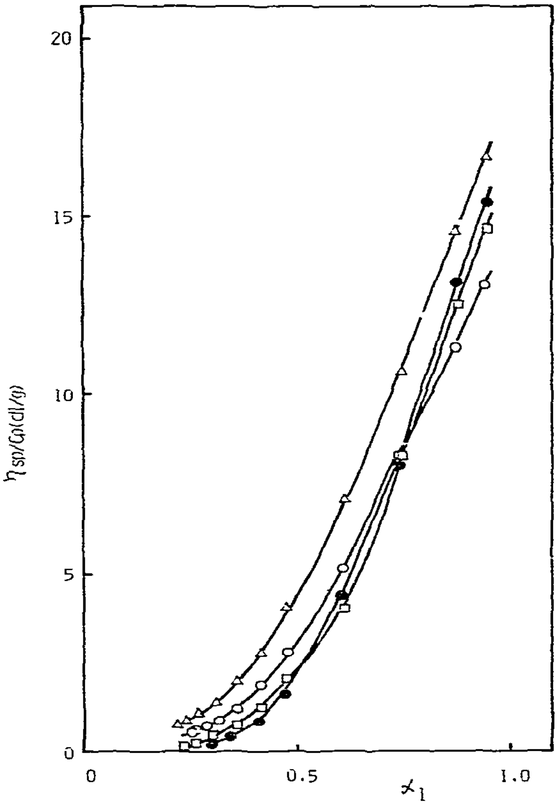

Fig. 4. The reduced viscosity of $(MA\text{-}St)_n$ in aqueous salt solution at $I = 0.09$ and 25°C. The symbols, MW and c_p the same as in fig. 3.

Anyway, the pH-induced conformational transitions observed in the various salt solutions used here are similar to those in the NaCl solution.

3.1.2. Temperature dependence of $[\eta]$ in aqueous NaCl

Dependence of $[\eta]$ of $(MA\text{-}St)_n$ on degree of neutralization α'_1 of the primary carboxyl groups was previously shown to reflect the transition [11]. The unper-

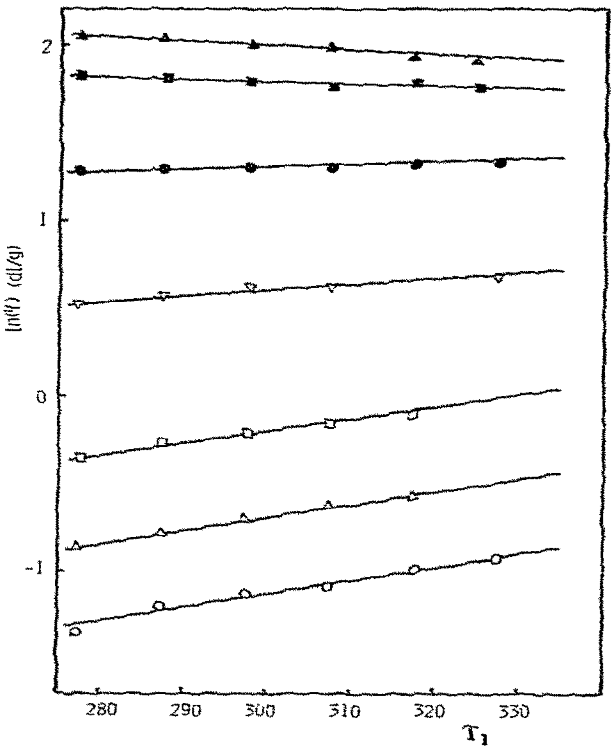

Fig. 5. Temperature dependence of $[\eta]$ of $(\text{MA-St})_n$ of MW = 613 000 in aqueous NaCl of 0.09 M: $\alpha_1' = 0.10$ (○), 0.20 (△), 0.30 (□), 0.45 (▽), 0.60 (●), 0.75 (▲) and 0.90 (■).

turbed RMS radius of dimension $\langle s_0^2\rangle^{1/2}$ and the apparent electrostatic interaction parameter B were both found to reflect the transition.

Here, the temperature dependence of $[\eta]$ of the fractionated $(\text{MA-St})_n$ of MW = 613 000 was studied in a temperature range of 5–55°C. In fig. 5, $[\eta]$ is shown to be almost proportional to temperature at each α_1'. The temperature coefficient $\mathrm{d}\ln[\eta]/\mathrm{d}T$ is 0.0077 ± 0.0003 (K^{-1}), irrespective of α_1', for the compact form ($\alpha_1' = 0.1$–0.3), but it is negative for the coiled form at $\alpha_1' = 0.90$. A plot of the coefficient against α_1' shown in fig. 6 seems to reflect its conformational transition as did the plots of $\langle s_0^2\rangle^{1/2}$ and the interaction parameter B. Recent studies on the temperature coefficient of viscosity of hyaluronic acid, which also undergoes a conformational transition upon ionization, have examined the similar change of the coefficient to $(\text{MA-St})_n$, and Cleland has interpreted such a change in sign in terms of transition between two segmental states [21]. The temperature coefficient of viscosity of $(\text{MA-St})_n$ may be related to the transition of a segmental state between the g- and c-forms. It is an important quantity for studies on the transition mechanism.

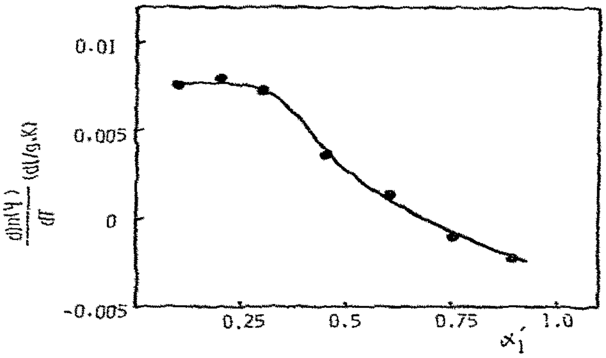

Fig. 6. The temperature coefficient of viscosity of $(\text{MA-St})_n$ calculated from the data in fig. 5.

3.1.3. Thermodynamic parameters of the transition

The standard free energy change, ΔG_t^0, for the transition of $(\text{MA-St})_n$ was previously determined from the pH-titration data in aqueous NaCl, and also ΔH_t^0 was calculated from the calorimetric measurements at ionic strength $I = 0.03$ and 25°C. Here, the calorimetric data of the transition at $I = 0.03$ are obtained at various temperatures. The results are shown in fig. 7, in which the anomalous behavior of ΔH_{app} is found at each

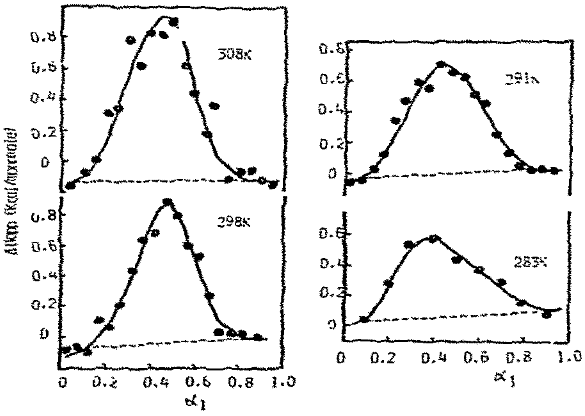

Fig. 7. The apparent dissociation heats ΔH_{app} of $(\text{MA-St})_n$ of MW = 250 000 in aqueous NaCl of 0.03 M. The broken lines are the same meaning as in fig. 5 in ref. [12].

Table 1
Thermodynamic parameters of the transitions of $(\mathrm{MA\text{-}St})_n$ and $(\mathrm{MA\text{-}BVE})_n$ in aqueous salt solution

Material	T (K)	I	ΔG (cal/monomole)	ΔH (cal/monomole)	ΔS (cal/monomole·K)	$\Delta\gamma_p$ (cal/monomole·K)	σ
$(\mathrm{MA\text{-}St})_n$	283	0.03		210	−0.41		
	291	0.03		280	−0.17		
	298	0.03	330*	340	0.04	9	0.13±0.08
	308	0.03		430	0.33		
$(\mathrm{MA\text{-}BVE})_n$	298	0.20		<0	<0		
	298	0.04		−345	−2.2		
	298	0	330	−560	−3.0	33**	
	318	0		93	−0.7		
benzene [33]	298	0	4610	580	−13.5	57–70	
butane [34]	298	0	5900	−800	−23	65–80	

* Temperature dependence of ΔG is very little.
** Calculated from the data at 298 K and 318 K.

temperature. ΔH_t^0 determined by the same method as in the previous report [12] was almost proportional to temperature in its range of 10–35°C, and the difference $\Delta\gamma_p$ between specific heats for the compact and coil forms could be determined.

The σ-value was estimated from the calorimetric data at 25°C as follows:

$$\sigma^{1/2} = \Delta H_t^0 / \Delta H_{\mathrm{vH}} , \qquad (1)$$

where ΔH_{vH} is the van 't Hoff enthalpy of the transition, which was calculated from the previously reported apparent fraction of the c-form f_c [12]. The relation between ΔH_{vH} and f_c is:

$$\Delta H_{\mathrm{vH}} = 4RT^2(\partial f_c/\partial T)_{f_c=0.5}$$
$$= 4RT^2[(\partial f_c/\partial \alpha_1)(\partial \alpha_1/\partial T)]_{f_c=0.5} \cdot \qquad (2)$$

We used $\partial f_c/\partial \alpha_1 = 1.74$ (from fig. 4 in ref. [12]) and $\partial \alpha_1/\partial T = (0.75 \pm 0.35) \times 10^{-3}$/K (from fig. 1 in ref. [12]).

Values of all parameters obtained here are summarized in table 1, where values for the n-butyl vinyl ether copolymer (MA-BVE) are compared [22–23].

3.1.4. Interaction with AO

Interaction of AO with $(\mathrm{MA\text{-}St})_n$ was previously studied by means of spectroscopic and pH-titration methods [20]. The absorption spectrum of AO depended on c_p/c_d (polymer to dye concentration ratio) and the copolymer conformation. Dilution of AO along the polymer chain was remarkable for the compact form, but scarce for the coil form. In order to estimate hydrophobicity of the compact form quantitatively, here, interaction of AO with the compact form $(\mathrm{MA\text{-}St})_n$ was investigated in detail. Spectroscopic titration data at 492 nm of the copolymer at $\alpha_1 = 0.27$ ($\alpha_1' = 0.20$) and $I = 0.143$ are shown in fig. 8 in a range of $\alpha_1 c_p/c_d$ less than 2, where the free AO and bound aggregates of AO are assumed to exist. From the results, the binding site of AO seems to be the charged carboxyl group. Equilibrium constant of binding of AO to the site is about 5.8×10^5 according to Schwarz's method [24]. On the other hand, in a range of $\alpha_1 c_p/c_d$ larger than 3, the remarkable dilution effect can be observed as an appearance of bound monomeric AO band at 502.5 nm [20]. Extrapolation of a plot of the molar extinction coefficient at the peak against $(\alpha_1 c_p/c_d)^{-1}$ to $c_p \to \infty$ can give the dimerization free energy of AO, which relates to the hydrophobicity of the copolymer [19,25]. Such an extrapolation is shown in fig. 9, from which the free energy at $I = 0.143$, $\alpha_1 = 0.27$ and $c_d = 1.15 \times 10^{-5}$ mole/l is found to be about −2.1 kcal/mole at 25°C. The free energy is −10.7 kcal/mole in water at 25°C [26].

Fractions of the bound dye in monomeric, dimeric and aggregate forms to the hydrophobic polymers are interpreted theoretically by the linear Ising model with the first, second and third nearest neighbour interac-

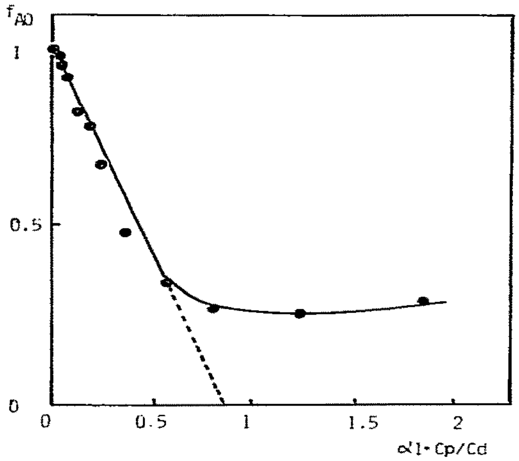

Fig. 8. Free fraction of AO, f_{AO}, in aqueous 0.143 M NaCl of $(\text{MA-St})_n$ of MW = 360 000 at $\alpha_1 = 0.27$, $c_d = 1.15 \times 10^{-5}$ mole/l and 25°C. Number of binding sites of AO per charged carboxyl groups calculated by Schwarz's method [24] is about 0.90.

tions [27,28]. Vitagliano et al. have attempted to obtain such fractions of bound AO to some acidic polyelectrolytes [19,25,29], but their method had to use some assumptions. We did not make the diagram of dependence of each fraction against $\alpha_1 c_p/c_d$.

Anyway, the compact conformation of $(\text{MA-St})_n$ has the hydrophobic domain being able to disperse the monomeric AO.

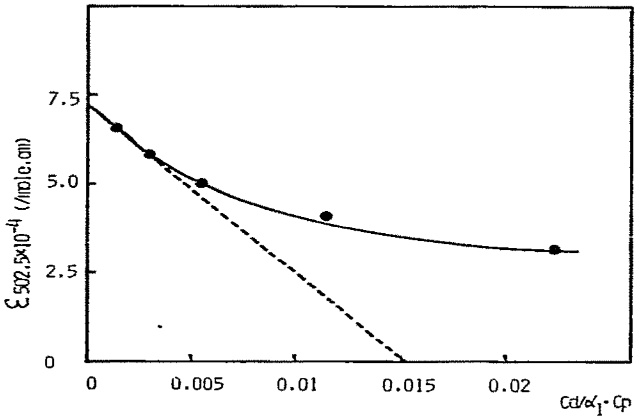

Fig. 9. $\Delta\epsilon_{502.5}$ versus $c_d/\alpha_1 c_p$ of $(\text{MA-St})_n$. MW, α_1, c_d, I and T the same as in fig. 8. The stacking coefficient q_1 of AO = 33 [19,27].

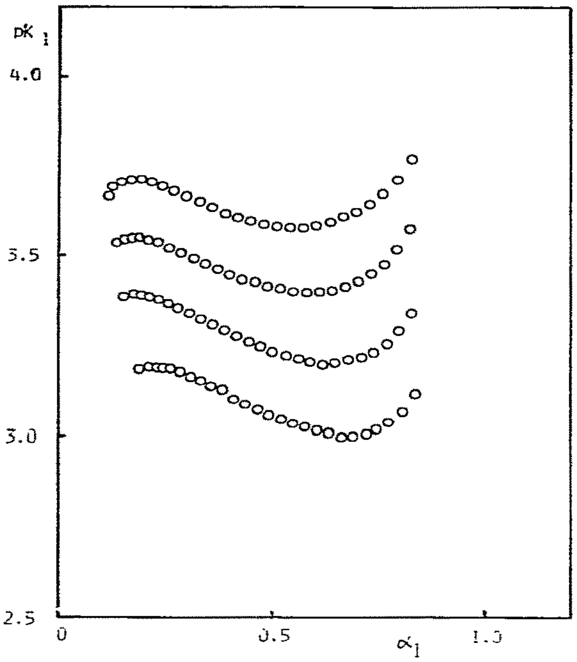

Fig. 10. The modified pH-titration curves of unfractionated $(\text{MA-MSt})_n$ of $c_p = 1.37 \times 10^{-2}$ monomole/l in aqueous NaCl at 25°C: I = 0.27, 0.09, 0.03, 0.009 from bottom to top.

3.2. Conformational transition of $(\text{MA-MSt})_n$

3.2.1. Potentiometric titration and viscosity

$(\text{MA-MSt})_n$ has a well defined equivalent point at $\alpha_1 = 1.0$. The modified pH-titration diagram of unfractionated sample (low MW copolymer was eliminated through dialysis) in aqueous NaCl is shown in fig. 10, where the conformational transition is clearly found at each ionic strength. The pH-titration of a fractionated sample of MW = 71 000 showed the similar transition. Values of pK_1^0 (at $\alpha_1 = 0$) are considerably smaller than those for $(\text{MA-St})_n$. Determination of exact values of pK_1^0 and of the transition free energy ΔG_t^0 at zero charge is difficult from the results in fig. 10. Back titration with HCl has to be achieved. Such data will be soon reported. Viscometric data showed that the transition is also from the compact to extended form, but the acid compact form is not so compact as for $(\text{MA-St})_n$.

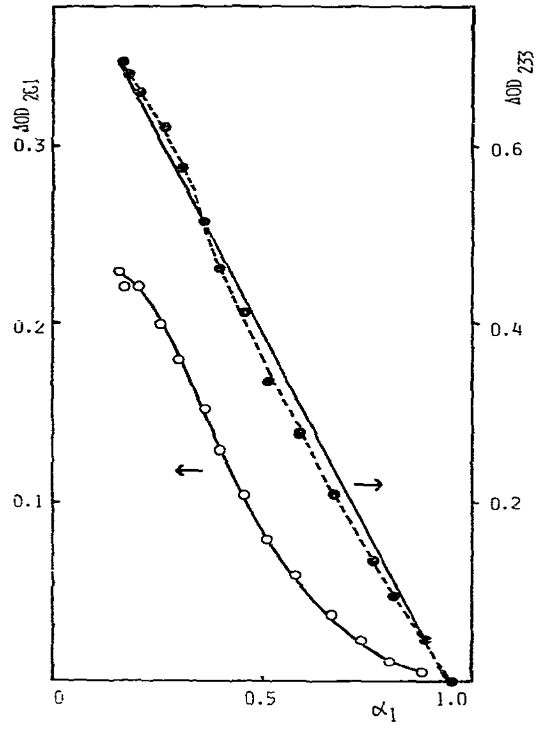

Fig. 11. $\Delta\epsilon_{261}$ and $\Delta\epsilon_{233}$ of $(\text{MA-MSt})_n$ of MW = 71 000 in aqueous NaCl of 0.09 M. The dotted lines may show that the plot of $\Delta\epsilon_{233}$ indicates the conformational transition.

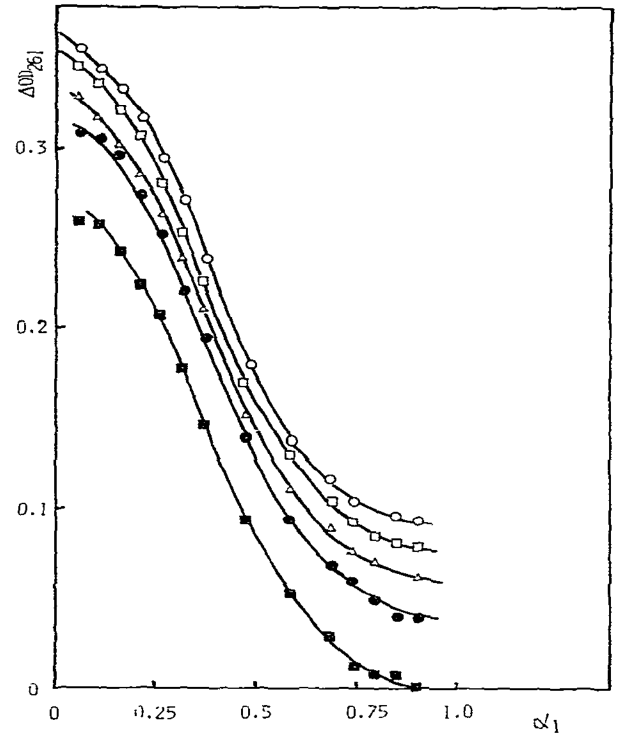

Fig. 12. Temperature dependence of $\Delta\epsilon_{261}$ of $(\text{MA-MSt})_n$ of MW = 71 000 and $c_p = 1.58 \times 10^{-2}$ monomole/l in aqueous NaCl at $I = 0.009$: T = 5°C, 15°C, 25°C, 35°C and 45°C from bottom to top.

3.2.2. Optical titration

The difference spectrum of the copolymer was obtained, the coil form copolymer being used as a reference. The spectrum in aqueous NaCl has some peaks at 233 nm, 261 nm and so on. The peak at 233 nm is concerned with the charged carboxyl groups and that at 261 nm with the phenyl ones. In fig. 11, plots of $\Delta\epsilon_{233}$ and $\Delta\epsilon_{261}$ of the fractionated copolymer against α_1 are shown at $I = 0.03$ and 25°C, where $\Delta\epsilon$ means the difference in molar extinction coefficient. The plot of $\Delta\epsilon_{261}$ is in a reversed sigmoidal fashion, and may show the transition. The compact form is understood to be more unstable than that of $(\text{MA-St})_n$ by comparing with the previous plot for $(\text{MA-St})_n$ [12]. However, the plots of $\Delta\epsilon_{233}$ seems to be almost proportional to α_1. In fig. 12, the same plots at 261 nm are shown at various temperatures and $I = 0.009$, where the coil form at 5°C is used as a reference. The compact form at $I = 0.009$ may be very unstable at all temperatures studied.

3.2.3. Dilatometric measurements

Dilatometric method is powerful for study of the conformational transition. In fig. 13, the volume change ΔV of monosodium salt of $(\text{MA-MSt})_n$ observed in back titration in aqueous NaCl of 0.009 M with HCl is shown. The slope in the central region includes the volume change ΔV_t due to the conformational transition from the coil to compact form. Values of ΔV_t are −1.6 and −0.8 ml/monomole at $I = 0.009$ and 0.09, respectively. It should be noted that ΔV_t of $(\text{MA-St})_n$ was reported to be positive at $I = 0.01 \sim 0.09$ [11]. Negative ΔV_t of $(\text{MA-MSt})_n$ seems to indicate the importance of other factors rather than the hydrophobic force between the phenyl groups for stabilization of the compact form of $(\text{MA-MSt})_n$.

3.2.4. Interaction of AO

Interaction of AO with the copolymer was studied

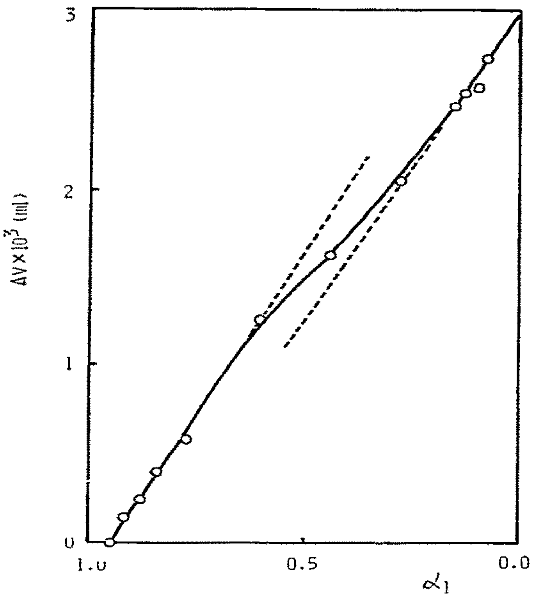

Fig. 13. Volume change of monosodium salt of $(\text{MA-MSt})_n$ of MW = 71 000 during back titration with HCl at 25°C. NaCl concentration, 0.009 mole/l and initial $\alpha_1 = 0.90$. The titration method the same as in figs. 7 and 8 in ref. [11].

at 25°C. The dilution effect of AO in the compact form copolymer in aqueous NaCl was substantial. The absorption maximum of the bound monomeric AO band was at about 502 nm. However, in the coiled conformation, the dilution effect was hardly found. At $c_p/c_d = 284$, $I = 0.009$ and 25°C, the bound monomeric AO to $(\text{MA-MSt})_n$ can hardly be found even at $\alpha_1' = 0.20$. From the results, also, the compact form of the copolymer is understood to be very unstable.

4. Discussion

All results obtained in this paper show the conformational transitions of both the hydrophobic copolymers and the existence of the compact forms in the acidic pH region. From the slight dependence of the reduced viscosity of $(\text{MA-St})_n$ in the compact form on c_p, the form seems to be near the θ-state. The viscometric expansion factor α_η defined by $[\eta]/[\eta]_\theta = \alpha_\eta^3$ was calculated from the $[\eta]$-values of $(\text{MA-St})_n$ in fig. 5 and the $[\eta]_\theta$-values estimated previously [11]. The value of α_η is 1.8 ± 0.2 for the compact form at 25°C. $(\text{MA-St})_n$ in the compact form is not completely at the θ-state, but may be not so far from the θ-state. The temperature coefficients of viscosity of vinyl polyelectrolytes are known to be positive, especially in the neighbourhood of the θ-state [30]. Therefore, the coefficient in the compact form is positive irrespective of α_1. What is a main cause of the negative temperature coefficient of viscosity of $(\text{MA-St})_n$ in the coil form? The maxima of reduced viscosity of $(\text{MA-St})_n$ are found at $\alpha_1 = 1.0$ in all cases (fig. 2 and ref. [13]). Also, conductivity data of $(\text{MA-St})_n$ at $\alpha_1 = 1.0$ fit to Manning's conductivity theory of a rod-like model [31]. Then the conformation of $(\text{MA-St})_n$ in the extended form seems to be approximated by the wormlike cylindrical model, as the charged polysaccharides. The negative temperature coefficient of viscosity of $(\text{MA-St})_n$ at $\alpha_1 = 0.90$ may be concerned with a negative temperature coefficient of the Kratky-Porod persistence length.

The value of $\Delta\gamma_p$ is important for discussing the compact form. For the transition of $(\text{MA-St})_n$, it is only 15% of that for the transfer of benzene to an aqueous medium, although the corresponding value of $(\text{MA-BVE})_n$ is about half of $\Delta\gamma_p$ for butane. A butyl group is more flexible than the phenyl group, and it can be more densely folded than the latter in the maleic acid copolymer in the compact form. The compact form of $(\text{MA-St})_n$ is considerably expanded, and, therefore, an important interaction may be the short range interaction. The equilibrium of the transition, the interaction of AO with the compact form copolymer and the kinetics of transition can be interpreted in terms of the linear Ising model with the first and second, and third (if necessary), interactions. $(\text{MA-MSt})_n$ has a more nonflexible back-bone structure than $(\text{MA-St})_n$, and its compact form is more unstable than that in the latter.

By the way, a relation between ΔH_t^0 and ΔS_t^0 was found to be as follows: $\Delta H_t^0 = \alpha_c + T_c \Delta S_t^0$, where $\alpha_c = 325$ cal/mole and $T_c = 300$ K. For $(\text{MA-BVE})_n$ the compensation plot [32] gives the same line. $(\text{MA-St})_n$ is considered to change the conformation in a similar style to $(\text{MA-BVE})_n$ and the transition free energy depends scarcely on the ionic strength and temperature due to the compensation effect.

Acknowledgements

One of the authors (S.S.) is indebted to Prof. J.A. Schellman for his critical comments. Also, the technical assistance of Dr. T. Okuda is gratefully acknowledged.

References

[1] J.C. Leyte and M. Mandel, J. Polym. Sci. Phys. Ed. 2 (1964) 1879.
[2] M. Mandel, J.C. Leyte and M.G. Stadhouder, J. Phys. Chem. 71 (1967) 603.
[3] V. Creszenzi, Adv. Polym. Sci. 5 (1968) 358.
[4] C. Braud, G. Müller, J.C. Fenyo and E. Sélègny, J. Polym. Sci., Chem. Ed. 12 (1974) 2767.
[5] E. Okamoto and Y. Wada, J. Polym. Sci., Phys. Ed. 12 (1974) 2413.
[6] P.L. Dubin and U.P. Strauss, J. Phys. Chem. 71 (1967) 2757.
[7] P.L. Dubin and U.P. Strauss, J. Phys. Chem. 74 (1970) 2842.
[8] P.L. Dubin and U.P. Strauss, in: Polyelectrolytes and their application, eds. A. Rembau and E. Sélègny (Reidel, Dordrecht, 1975) p. 3.
[9] U.P. Strauss and G. Vesnaver, J. Phys. Chem. 79 (1975) 1558, 2426.
[10] U.P. Strauss and M.S. Schlesinger, J. Phys. Chem. 82 (1978) 571.
[11] N. Ohno, K. Nitta, S. Makino and S. Sugai, J. Polym. Sci., Phys. Ed. 11 (1973) 413.
[12] T. Okuda, N. Ohno, K. Nitta and S. Sugai, J. Polym. Sci., Phys. Ed. 15 (1977) 749.
[13] N. Ohno, K. Nitta and S. Sugai, Kobunshi Kagaku 28 (1971) 82.
[14] P.A. Demechenko and V.P. Voiko, Vyskomol. Soedin 15 (1973) 2330.
[15] S. Sugai, N. Ohno and K. Nitta, Macromolecules 7 (1974) 96.
[16] N. Ohno, K. Nitta and S. Sugai, J. Polym. Sci., Phys. Ed. 16 (1978) 513.
[17] R.B. Seymour and D.P. Garner, Polymer 17 (1976) 21.
[18] R. Endo, T. Hinokuma and M. Takeda, J. Polym. Sci. A-2, 6 (1968) 665.
[19] V. Vitagliano, L. Costantino and A. Zagari, J. Phys. Chem. 77 (1973) 204.
[20] N. Ohno, J. Chem. Soc. Japan (1978) 1137.
[21] R.L. Cleland, Biopolymers 18 (1979) 1821.
[22] V. Crescenzi, F. Quadrifoglio and F. Delben, J. Polym. Sci. C 39 (1972) 241.
[23] V. Crescenzi, in: Polyelectrolytes, ed. E. Sélègny (Reidel, Dordrecht, 1974) p. 115.
[24] G. Schwarz, Eur. J. Biochem. 12 (1970) 442, 454, 461.
[25] V. Vitagliano and L. Costalino, J. Phys. Chem. 74 (1970) 197.
[26] V. Zanker, Z. Phys. Chem. 199 (1952) 225.
[27] S. Lifson, J. Chem. Phys. 40 (1964) 3705.
[28] J.A. Schellman, Israel J. Chem. 12 (1974) 219.
[29] V. Vitagliano, L. Constantino and R. Sartorio, J. Phys. Chem. 80 (1976) 959.
[30] H. Eisenberg and D. Woodside, J. Chem. Phys. 36 (1962) 1844.
[31] G.S. Manning, J. Phys. Chem. 79 (1975) 262.
[32] R. Lumry and S. Rajender, Biopolymers 9 (1970) 1125.
[33] G. Nemethy and H.A. Scheraga, J. Chem. Phys. 36 (1962) 3401.
[34] C. Tanford, The hydrophobic effect (John Wiley & Sons, New York, 1973).