Studies on conformational transition of the maleic acid copolymer with styrene in aqueous salt solution by derivative spectroscopy

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The first and second derivative spectra for the optical absorption of maleic acid copolymer with styrene were measured in aqueous 0.09 M NaCl at 25°C within a wavelength range of 240–280 nm, where contributions of groups, other than the phenyl ones, to the second derivatives seem to be negligible. The normal absorption spectra in the range were composed of seven absorption bands and the peak position of each band was determined from the derivative spectra. The peak position of the coiled form copolymer was found to shift to the shorter wavelength range compared with the compact form of the copolymer. From the extent of such blue shifts for each peak, the environment near the phenyl groups in the compact copolymer form was concluded to be similar to aqueous ethanol (40–50 vol%), by comparing with the derivative spectra for *N*-acetyl-L-phenylalanine in a water–ethanol mixture. However, the first and second derivative spectra and various first derivative spectra were found to have 12 isoderivative points, and all the bands of the different spectra displayed the two-state character of the transition. The degree of transition was determined from the change in the peak height of each band and this coincided well with the pH-titration data.

Keywords Spectroscopy; transition; conformational studies; maleic acid; styrene; copolymer

INTRODUCTION

Copolymers of maleic acid with hydrophobic monomers have been found to undergo a conformational transition from the compact to extended form upon the ionization of the primary carboxyl groups[1–4], and the two-state character of the transition has been assumed[5]. A recent study on the transition of the maleic acid copolymer with butyl vinyl ether displayed its two-state character by means of pH-titration[6].

We have studied the pH-induced conformational transition of maleic acid copolymer with styrene $(MA\text{–}St)_n$, and have found from various absorption data at 269 nm that the compact form is stabilized by a hydrophobic interaction between the buried phenyl groups and the residues that are exposed after the transition[7–9]. The midpoint of transition, determined from the pH-titration results, coincided well with that determined from the optical results, and the two-state mechanism of the transition was assumed.

However, absorption at 269 nm for $(MA\text{–}St)_n$ comprises two terms: (1) perturbation of the phenyl groups produced by change in the charge on the nearby carboxyl group and (2) change of the environment near the phenyl groups. The first term was assumed to be proportional to the degree of ionization, α, for carboxyl groups and the hypothetical values for the different absorption coefficients at 269 nm for both the compact and coiled forms were assumed over the whole range of α studied. The degree of the transition of the copolymer determined from the optical method, under such an assumption, might result in considerable ambiguity. The degree of transition from the pH-titration data under the two-state assumption has been known to include similar ambiguity[9].

Second derivative spectra for the absorption of globular proteins have recently shown complete separation of the absorption of phenylalanine from that of other residues such as tyrosine and tryptophan, and has been reported to reflect the environment near the phenyl groups and the conformational transition[10,11]. The derivative spectra of $(MA\text{–}St)_n$ in the wavelength region 250–280 nm, may completely separate the absorption of the phenyl groups from that of charged or uncharged carboxyl groups and give some important information about the transition.

In this paper, studies on the derivative spectra of the optical absorption of $(MA\text{–}St)_n$ in aqueous NaCl (0.09 M) at 25°C are described. The transition profile for the copolymer is discussed by using the separated absorption bands of the phenyl groups in the derivative spectra. Also, the hydrophobic environment near the phenyl groups in the compact form of the copolymer was investigated by comparing the derivative spectra for a monomer analogue, *N*-acetyl-L-phenylalanine, in a water–ethanol mixture.

EXPERIMENTAL

Materials

$(MA\text{–}St)_n$ of molecular weight of 9.4×10^5 was prepared by a method described previously[7]. Two salt-free stock

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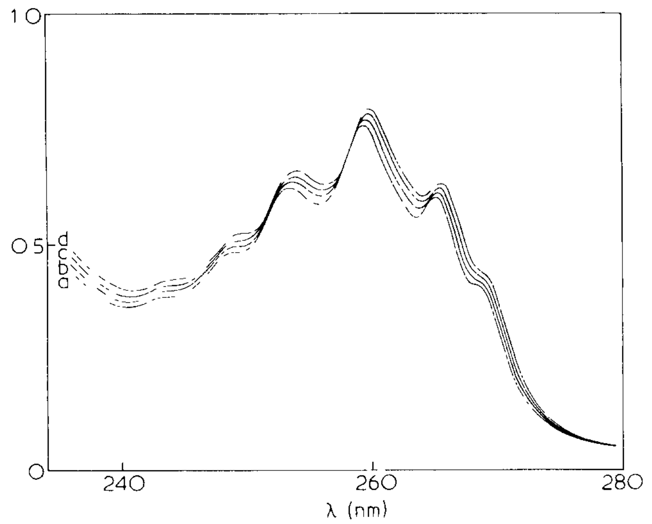

Figure 1 Absorption spectra for $(MA{-}St)_n$, 5.29 x 10^{-3} mmol l^{-1} in aqueous NaCl (0.09 M) at 25°C. α_1: 0.80, (a); 0.58, (b); 0.41, (c); and 0.27, (d)

solutions were prepared, whose degrees of neutralization for the primary carboxyl groups were $\alpha_1 = 0.20$ and 0.80 respectively. The polymer concentrations (c_p) of the stock solutions were determined potentiometrically to be about 4.8×10^{-2} mmol l^{-1}. Aqueous NaCl solutions of $(MA{-}St)_n$, of ionic strength $I = 0.09$, $c_p = 6{-}3 \times 10^{-3}$ mmol l^{-1} and degree of ionization of the primary carboxyl group ($\alpha_1 = 0.27{-}0.80$), were made from stock solutions. The monomer analogue, *N*-acetyl-L-phenylalanine, was purchased from Nakarai Chem. Co., Kyoto, and its concentration was determined spectrophotometrically using the molar absorption coefficient at 258.0 nm 195 (M^{-1} cm^{-1})[11,12]. All other chemicals were special grade from the Nakarai Chem. Company.

Methods

The normal absorption spectrum was obtained at 25 C with a Union spectrophotometer SM 401, which was controlled with a microcomputer Sord M 222, with a spectral band width of 0.5 nm, with a response time of 0.1 s and a scan speed of 1 nm s^{-1}. Absorbance $A(\lambda)$ was recorded for each 0.1 nm and accumulated 25–100 times with the microcomputer. The first and second derivative spectra were defined as follows:

$$\frac{dA(\lambda)}{d\lambda} = \frac{1}{2d}[A(\lambda + d) - A(\lambda - d)](nm^{-1}) \qquad (1)$$

$$\frac{d^2A(\lambda)}{d\lambda^2} = \frac{1}{d^2}[A(\lambda + d) - 2A(\lambda) + A(\lambda - d)](nm^{-2}) \qquad (2)$$

The S/N ratio of the derivative spectrum calculated by equations (1) or (2) was not good and a smoothing process was needed. Once smoothed, spectrum $B_1(\lambda)$ for the spectrum $B_0(\lambda)$ $\left(= \frac{dA(\lambda)}{d\lambda} \text{ or } \frac{d^2A(\lambda)}{d\lambda^2}\right)$ was defined as:

$$B_1(\lambda) = \frac{1}{4}[B_0(\lambda + d) + 2B_0(\lambda) + B_0(\lambda - d)] \qquad (3)$$

and the twice smoothed spectrum $B_2(\lambda)$ as:

$$B_2(\lambda) = \frac{1}{16}[B_0(\lambda + 2d) + 4B_0(\lambda + d) + 6B_0(\lambda) + 4B_0(\lambda - d) + B_0(\lambda - 2d)] \qquad (4)$$

and so on, where λ is expressed in nm and d (= 0.1 nm) is a sampling interval). The more often the smoothing process was repeated, the better the S/N ratio the worse was the expected spectral resolution, and the number of smoothing times was determined in connection with the band width of the spectrophotometer. Here, both the derivative spectra were smoothed 12 times. The extent of the blue shift of the peak or zero position of the spectrum could be determined within a wavelength accuracy of less than 0.1 nm.

We attempted to separate the derivative spectra into 7 components in the wavelength region of 240–280 nm, and each of them was assumed to be in the Gaussian form. The characteristic parameters of the Gaussian curve were calculated to get the fitted spectra using an electronic computer HITAC M 180.

pH measurements were made with a Horiba pH-meter M 7.

RESULTS

The normal absorption spectra of $(MA{-}St)_n$ (5.29 x 10^{-3} mmol l^{-1}) in aqueous NaCl (0.09 M at 25°C are shown with four α_1's in *Figure 1*, where the spectrum is not found to depend on α_1 to any great extent. Six peaks are clearly found in the region from 240–280 nm for both the compact and the coiled forms, and a blue shift of 0.3–1.0 nm is observed at each peak, at high α_1. Such a blue-shifted spectrum for the coiled form was previously shown[8] from the different spectra measurements. When the absorption spectrum shifts to the shorter wavelength region with little change in the spectral shape as the degree of ionization increases, the first derivative spectrum corresponds to the difference between the α_1 values of the two solutions, which are infinitely small. The position of each peak can be determined from the zero position of the first derivative spectrum. In *Figure 2*, the first derivatives of the absorption of $(MA{-}St)_n$ in aqueous NaCl are indicated by the three α_1's. The spectrum at each α_1 resembles those shown previously with reference to the coiled form[8,9]. Also, all of the derivatives are found to be very similar to the first derivative spectra of *N*-acetyl-L-phenylalanine in aqueous NaCl (0.09 M), which is shown in *Figure 3*.

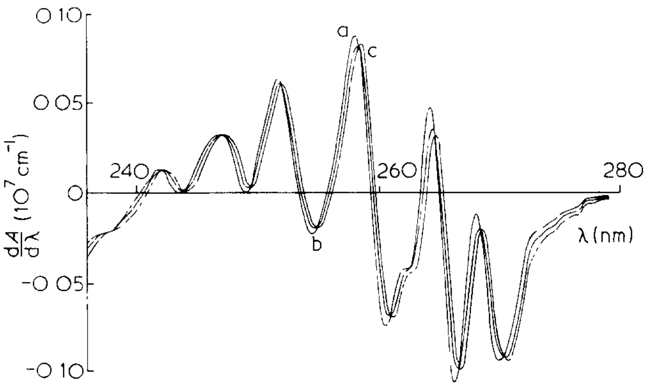

Figure 2 First derivative spectra for $(MA{-}St)_n$ 5.29 x 10^{-3} mmol l^{-} in aqueous NaCl (0.09 M) at 25°C. α_1: 0.80, (a); 0.52, (b) and 0.27, (c)

The wavelengths at the zero position in the first derivative spectra are listed and compared with the corresponding wavelengths of the analogues in *Table 1*. The peak for the compact form shifts to the longer wavelength region compared with the corresponding peak for the coiled form. By comparing the results for the analogue, the phenyl groups in the compact form can be concluded to be in the more hydrophobic domain than that in the coiled form.

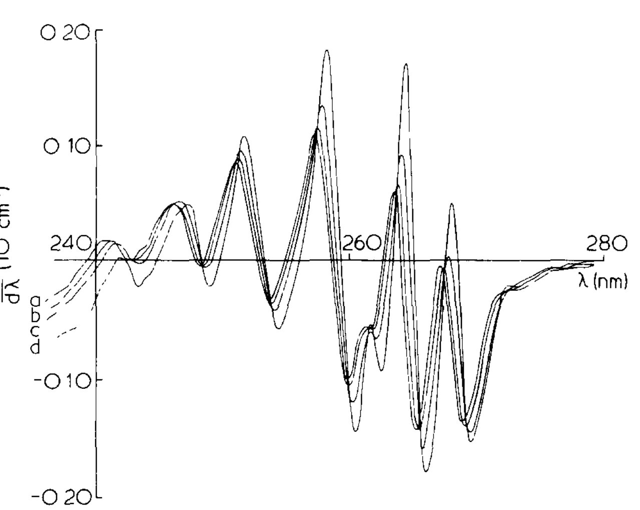

Figure 3 First derivative spectra for *N*-acetyl-L-phenyl-alanine, 2.36×10^{-3} mole l^{-1} in aqueous ethanol at 25°C. Ethanol content (vol %): 0, (a); 20, (b); 40, (c) and 100, (d)

Difference spectra for the first derivatives with reference to an α_1 value of 0.27, were calculated by the computer. In *Figure 4* different spectra are shown, which have 12 isoderivative points in the region 240–280 nm. The isoderivative points are also found in *Figure 2*. The existence of such points indicates that the conformational transition of $(MA\text{–}St)_n$ in aqueous NaCl is of a two-state type.

The difference between the spectrum for the first derivatives refers to the second derivative spectrum of absorption. The calculated second derivatives are indicated in *Figure 5*, where the isoderivative points can also be seen. Although absorption of other groups other than the phenyl groups is observed in the normal and first derivative spectra the wavelength region <240 nm, the contribution of these other groups is almost completely eliminated in *Figure* 5. The negative peak of the second derivative spectrum in a Gaussian form corresponds to the zero position of the first derivative and to the peak of the normal spectrum. In *Table 1*, the peak positions

Table 1(a) Wavelength at absorption peak for $(MA\text{–}St)_n$ in aqueous NaCl (0.09 M) and the extent of blue shift (Δλ) of the coiled form

c_p (mmol l^{-1})	Derivative spectrum	α_1	Wavelength (nm) Band I	II	III	IV	V	VI	VII
5.29×10^{-3}	First	0.27	244.2	–	253.9	259.8	–	265.5	–
	Second		243.3	248.5	253.2	259.5	262.8	265.7	269.4
	First	0.80	243.3	248.9	253.4	259.2	–	265.0	–
	Second		242.4	248.0	252.6	258.8	262.0	265.0	268.8
		Δλ =	0.9	0.5	0.45	0.65	0.8	0.6	0.6
3.00×10^{-3}	First	0.29	244.1	–	253.9	259.5	–	265.4	–
	Second		243.2	248.5	253.3	259.1	262.4	265.5	267.4
	First	0.80	243.2	248.8	253.2	259.0	–	265.0	–
	Second		242.5	248.0	252.5	258.7	262.0	265.0	268.8
		Δλ =	0.8	0.5	0.75	0.45	0.4	0.45	0.6

Table 1(b) Wavelength at absorption peak for *N*-acetyl-L-phenylalanine of 2.36×10^{-3} mol l^{-1} in water–ethanol mixture

Derivative spectrum	Ethanol (vol %)	Wavelength (nm) Band I	II	III	IV	V	VI	VII
First	0	–	247.9	252.5	258.4	–	264.3	–
	10	–	248.0	252.6	258.5	–	264.4	–
	20	–	248.2	252.8	258.7	–	264.5	–
	30	–	248.2	252.9	260.0	–	264.6	–
	40	–	248.3	253.1	260.0	–	264.8	–
	50	–	248.4	253.2	260.2	–	264.9	268.0
	100	–	248.4	253.4	260.4	–	265.1	268.2
	0*	–	247.8	252.5	258.4	–	264.2	268.5
Second	0	242.0	247.5	252.2	258.3	261.7	264.3	268.0
	10	242.0	247.5	252.3	258.3	261.8	264.4	268.1
	20	242.1	247.5	252.4	258.4	261.8	264.6	268.2
	30	242.1	247.5	252.5	258.6	261.8	264.7	268.3
	40	242.2	247.6	252.7	258.7	262.0	264.8	268.4
	50	242.2	247.7	253.1	258.8	262.0	264.9	268.6
	100	243.1	247.9	253.5	258.8	262.1	265.0	268.7
	0*	241.8	247.3	250.0	258.1	261.6	264.2	267.9

* Concentration of the analogue = 2.49×10^{-3} mol l^{-1}, 0.09 M NaCl solution

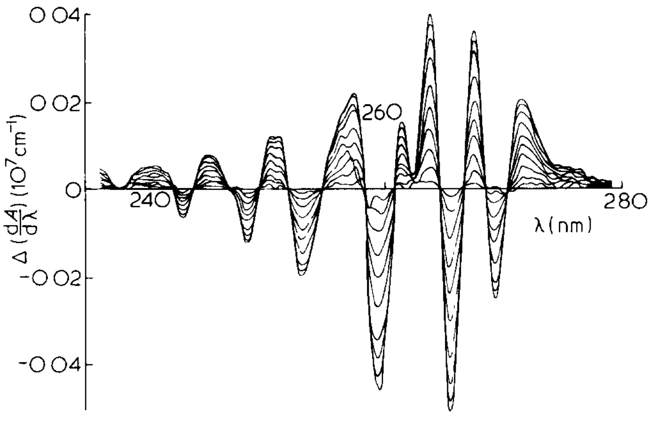

Figure 4 Difference spectra for the first derivatives of $(MA\text{–}St)_n$ 5.29×10^{-3} mmol l^{-1} in aqueous NaCl (0.09 M) at 25°C. The compact form at $\alpha_1 = 0.27$ was used as a reference. α_1: 0.31, 0.36, 0.41, 0.46, 0.52, 0.58, 0.63, 0.69, 0.75 and 0.80 from bottom to top at each peak

estimated from the second derivative spectra are also listed and compared with the zero positions of the first derivative spectra. The extent of the blue shift is calculated at various α_1's.

DISCUSSION

As the spectral shape of the absorption $A(\lambda)$ scarcely depends on α_1, the difference, $\Delta A(\lambda,\alpha_1)$, between the spectra at α_1 with reference to α_0 is:

$$\Delta A(\lambda,\alpha_1) = A(\lambda,\alpha_1) - A(\lambda,\alpha_0) = A(\lambda + \Delta\lambda) - A(\lambda)$$

$$= \frac{dA(\lambda)}{d\lambda}\Delta\lambda + \frac{1}{2}\frac{d^2A(\lambda)}{d\lambda^2}(\Delta\lambda)^2 + \ldots \qquad (5)$$

where $\Delta\lambda$ means the extent of the blue shift of the sample at α_1. The spectrum relates simply to the first derivative spectrum, in a limiting case, i.e. $\Delta\lambda \rightarrow 0$. Similarly, the second derivative spectrum is a limiting difference example of the first derivatives. The derivative spectrum has some advantages in analysing the optical absorption data: (1) the optical absorption bands of a chromophore can easily be separated from those of other ones; (2) the fine structure of the bands of the chromophore can be analysed from it, and so on.

Six absorption bands are found in the wavelength region 240–280 nm from the normal spectrum of $(MA\text{–}St)_n$, but one additional band has to be assumed around 262 nm from the derivatives. Here, the seven bands are styled 'Band I, II, III, IV, V, VI and VII' as shown in *Table 1*. N-acetyl-L-phenylalanine has the corresponding seven bands. If each band is approximated to be the Gaussian form, absorbance of $(MA\text{–}St)_n$ is:

$$A(\lambda) = \sum_{i=1}^{7} A_i \exp\left[-\left(\frac{\lambda - \lambda_i}{\Delta_i}\right)^2\right] + A_0(\lambda) \qquad (6)$$

where $A_0(\lambda)$ is the absorbance of groups other than the phenyl groups. At 240–280 nm, $d^2A_0(\lambda)/d\lambda^2$ can be assumed to be negligible, and the derivatives of $A(\lambda)$ are:

$$\frac{dA(\lambda)}{d\lambda} = -2\sum_{i=1}^{7}\left(\frac{\lambda - \lambda_i}{\Delta_i^2}\right)^2 A_i \exp\left[-\left(\frac{\lambda - \lambda_i}{\Delta_i^2}\right)^2\right] + \frac{dA_0(\lambda)}{d\lambda} \qquad (7)$$

and

$$\frac{d^2A(\lambda)}{d\lambda^2} = -2\sum_{i=1}^{7}\frac{1}{\Delta_i^2}\left[1 - 2\left(\frac{\lambda - \lambda_i}{\Delta_i}\right)^2\right] A_i \exp\left[-\left(\frac{\lambda - \lambda_i}{\Delta_i}\right)^2\right] \qquad (8)$$

in the region of wavelength longer than 240 nm. A_i (absorbance at peak), λ_i (wavelength at peak) and Δ_i (band width) are the characteristic parameters of the i-th band. If the i-th band is isolated from other bands, $A(\lambda)$ is simply expressed, around $\lambda = \lambda_i$, as:

$$A(\lambda) = A_i \exp\left[-\left(\frac{\lambda - \lambda_i}{\Delta_i}\right)^2\right] \qquad (9)$$

In such a case, $dA(\lambda_i)/d\lambda = 0$ and $d^2A(\lambda_i)/d\lambda^2$ has a minimum value at $\lambda = \lambda_i$. Band VI seems to be in such an isolated form as shown in *Table 1*, in which the wavelength at the zero position of the first derivative spectrum of Band VI coincides well with that from the minimum of the second derivative spectrum.

All of the characteristic parameters in equations (6)–(8) were estimated by means of the non-linear least square method to obtain the fitted curve to the second derivative spectrum at 240–280 nm. For both the compact and coiled forms, considerable agreement for the calculated curves with the spectra were obtained under the assumption of the seven Gaussian bands. In *Table 2*, the calculated parameters are listed for cases where $c_p = 5.29 \times 10^{-3}$ and 3.00×10^{-3} mmol l^{-1}. The position of Band VI coincides well with that estimated from the zero position of the first and the negative peak of the second derivative spectrum. Also, the table shows the blue shift of Band VI. By comparing the extent (0.45–0.6 nm) of the blue shift of Band VI (*Table 1(a)*) with the data for the analogue in water–ethanol mixture, the environment near the phenyl groups in the compact form of the copolymer is considered to be similar to aqueous medium of 40–50 vol% ethanol, but it is not as completely hydrophobic as the inner parts of the globular proteins. The results are consistent with the models for the compact conformation of the hydrophobic polyelectrolyte introduced previously by some authors[5,13].

The isoderivative points found in *Figures 2, 4* and *5* show the two-state character of the conformational

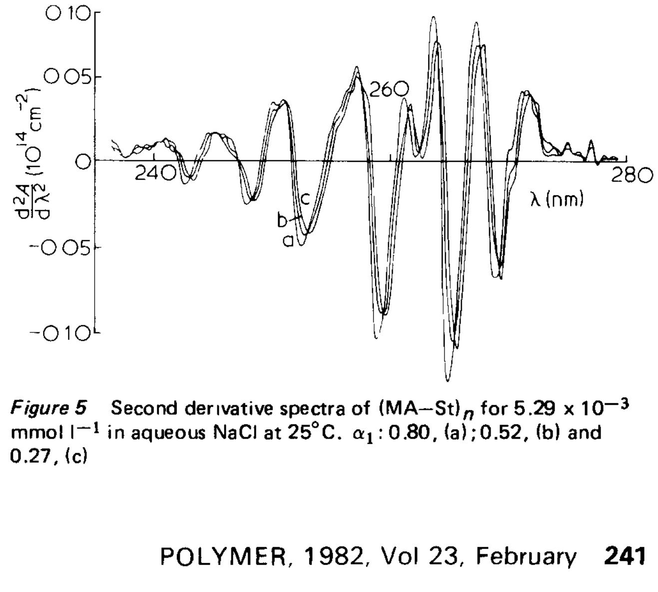

Figure 5 Second derivative spectra of $(MA\text{–}St)_n$ for 5.29×10^{-3} mmol l^{-1} in aqueous NaCl at 25°C. α_1: 0.80, (a); 0.52, (b) and 0.27, (c)

Table 2 Gaussian parameters for each absorption band of $(MA\text{–}St)_n$ in aqueous NaCl (0.09 M) by means of the non-linear least square method

α_1	Band	A_i	λ_i (nm)	Δ_i (nm)	α_1	Band	A_i	λ_i (nm)	Δ_i (nm)
(1) $c_p = 3.00 \times 10^{-3}$ mmol l^{-1}									
0.29	I	0.061	243.9	2.1	0.80	I	0.036	244.4	1.4
	II	0.154	248.1	2.2		II	0.135	248.0	2.0
	III	0.461	254.1	4.0		III	0.436	254.0	4.1
	IV	0.406	259.2	2.2		IV	0.394	259.0	2.2
	V	0.471	262.6	3.5		V	0.448	262.5	3.5
	VI	0.208	265.7	1.7		VI	0.219	265.4	2.0
	VII	0.332	268.8	3.2		VII	0.291	268.7	3.0
(2) $c_p = 5.29 \times 10^{-3}$ mmol l^{-1}									
0.27	I	0	244.5	–*	0.80**	I	–	–	–
	II	0.072	248.5	1.5		II	0.023	248.4	0.8
	III	0.307	254.2	3.4		III	0.228	253.4	3.3
	IV	0.164	259.2	1.7		IV	0.094	259.0	1.3
	V	0.404	261.4	3.9		V	0.398	260.4	4.6
	VI	0.231	265.7	1.8		VI	0.239	265.3	1.8
	VII	0.224	269.0	2.6		VII	0.206	268.7	2.4

* Δ_1 could not be estimated accurately

** Calculation was done under the assumption of existence of six bands

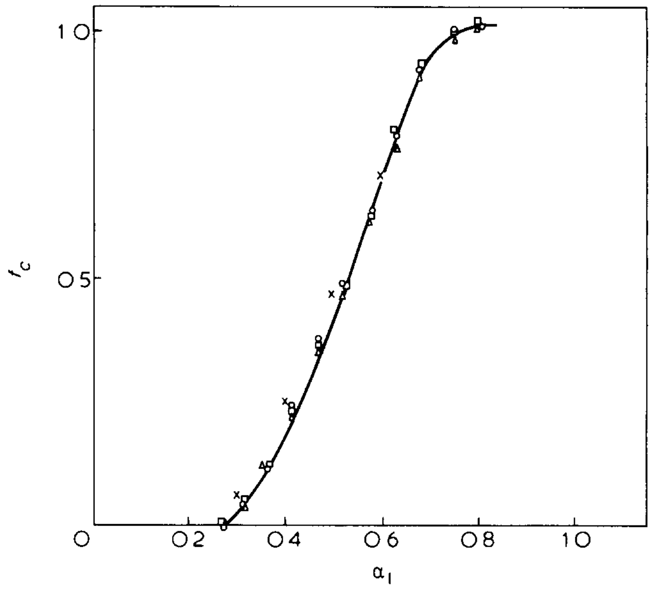

Figure 6 Dependence of f_c (fraction of residue in the coiled form) on α_1. Calculation was done under the assumption that $f_c = 0$ and 1.0 at $\alpha_1 = 0.27$ and 0.80, respectively. Wavelength: 253.1 nm (□), 265.4 nm (○) and 267.5 nm (△). (X), pH–titration results

transition of $(MA\text{–}St)_n$ in an aqueous medium. In *Figure 4* it is important to analyse the transition mechanism, where each band of the spectrum is clearly indicated. By use of the α_1-dependence of the peak height at each band, the degree of transition can be estimated as a function of α_1. In *Figure 6*, the fraction of the residue in the coiled form in $(MA\text{–}St)_n$ is given by calculating it from the negative peak at 253.1 nm (Band III), the negative peak at 265.4 nm (Band VI) and the positive peak at 267.5 nm (Band VII). The degrees of transition from the three bands agree very well with each other. In *Figure 6*, the degrees of transition from the pH-titration data calculated previously under the two-state assumption[9] are compared. The pH-titration data also coincide with the present data.

The conformational transition is therefore assumed to be a two-state type for $(MA\text{–}St)_n$ in aqueous NaCl.

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REFERENCES

1 Dubin, P. L. and Strauss, U. P. *J. Phys. Chem.* 1967, **71**, 2757
2 Dubin, P. L. and Strauss, U. P. *J. Phys. Chem.* 1970, **74**, 2842
3 Strauss, U. P. and Vesnaver, G. *J. Phys. Chem.* 1975, **79**, 1558
4 Strauss, U. P. and Schlesinger, M. S. *J. Phys. Chem.* 1978, **82**, 571
5 Dubin, P. L. and Strauss, U. P. 'Polyelectrolytes and Their Applications' (Eds. A. Renbaum and E. Selegny), Reidel, Dordrecht, 1975, p. 3
6 Strauss, U. P., Barbleri, B. W. and Wong, G. *J. Phys. Chem.* 1979, **83**, 2840
7 Ohno, N., Nitta, K., Makino, S. and Sugai, S. *J. Polym. Sci., Polym. Phys. Edn.* 1973, **11**, 413
8 Sugai, S., Ohno, N. and Nitta, K. *Macromolecules* 1974, **7**, 96
9 Okuda, T., Ohno, N., Nitta, K. and Sugai, S. *J. Polym. Sci., Polym. Phys. Edn.* 1977, **15**, 749
10 Balestrieri, C., Colonna, G., Giovane, A., Irace, G. and Servillo, L. *Eur. J. Biochem.* 1978, **90**, 433
11 Ichikawa, T. and Terada, H. *Biochim. Biophys. Acta* 1979, **580**, 120
12 Scheraga, H. A. 'Protein Structure', Academic Press, New York and London, 1961, p. 217
13 Sugai, S. and Ohno, N. *Biophys. Chem.* 1980, **11**, 387