Estimation of α and β Peptide Bonds in Thermal Poly(aspartic acid) by Potentiometric Titration
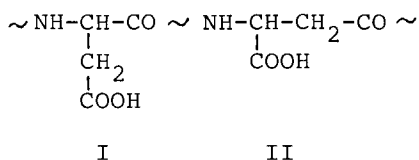
V. Saudek and J. Drobník

Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, 162 06 Prague 6, Czechoslovakia

Summary

A potentiometric titration method for the estimation of the configuration (α and β peptide bonds, I and II) of poly(aspartic acid) prepared by thermal polycondensation is presented. Experimental results of the potentiometric titration of various samples differing in the molar fraction of I and II are confronted with the results of ^{13}C NMR spectroscopy. Both methods lead to comparable results.

Introduction

Poly(Aspartic acid), poly(Asp), prepared by thermal polycondensation, and its derivatives are being studied as potential drug carriers, plasma expanders, models in the investigation of the fate of polymers in the body (KÁLAL et al. 1978) and in some other model studies (cf. PIVCOVÁ et al. 1981). Poly(Asp) is also regarded by some authors as a kind of a primitive prebiotic protein (proteinoid, FOX and DOSE 1977). It has been established that aspartic acid is linked in the main chain by the α (I) and β (II) peptide bonds:

$$\sim NH-CH-CO \sim NH-CH-CH_2-CO \sim$$
$$\underset{\underset{COOH}{|}}{\overset{|}{CH_2}} \qquad \underset{COOH}{|}$$

I   II

The mole ratio of I and II may be determined by ^{13}C NMR (PIVCOVÁ et al. 1981) and according to some preliminary results also by ^{1}H NMR spectroscopy (MATSUYAMA et al. 1980). An alternative method is potentiometric titration (henceforth PT) which is used by Harada and coworkers. Analysing this method (SAUDEK 1981), we have shown that its treatment of the experimental data was not correct. Erroneous values of pK of I and II and of their molar fraction were obtained by extrapolation of the PT data and by the evaluation of the inflection

point in the PT curve (KOKUFUTA et al. 1977). Con
sequently, the calculation of the molar fraction of II
based on these pK values (HARADA et al. 1978,
MATSUYAMA et al. 1980) also provided incorrect results.
We propose, in the present communication, another
approach.

Experimental

     The samples of poly(D,L-aspartic acid), poly(Asp),
were prepared by hydrolysis of poly(D,L-succinimide)
(product of the thermal polycondensation of aspartic
acid) under various pH. Their preparation and charac-
terisation will be described in detail elsewhere
(PIVCOVÁ et al.). The molar fraction of II and
intrinsic viscosity are given in Table I.
     PT was carried out with a Mettler automatic
titrator equipped with the Radiometer combined elec-
trode GK2402C under nitrogen atmosphere at 25°C. pH
scale was calibrated with Radiometer buffers pH 7.00,
4.01 and 2.68. About 20 mg of a polymer was dissolved
in 50 ml of 2 molal NaCl, 2.5 ml of 0.1 M NaOH was ad-
ded and the solution was titrated with 0.1 M HCl. The
titrant was added in such portions that the change of
pH was 0.04-0.1 unit, each portion 10 s after
$dpH/dt < 5 \times 10^{-4}$ pH unit/s. The exact concentration of
the polymers was determined by PT and elemental
analysis (N). The activity coefficient of H^+ ion in 2
molal NaCl was determined by titration of the solvent.

Data Treatment

     An equation describing the dependence of pH on
the degree of ionization (α) upon neglection of any
polymer effect has been derived (SAUDEK, 1981):

$$\alpha = \frac{K_1 k}{K_1 + H} + \frac{K_2(1-k)}{K_2 + H} \qquad (1)$$

$$\alpha = \frac{[Na^+] - [OH^-] + H}{C} \qquad (2)$$

where $[Na^+]$, $[OH^+]$, H, C, k, K_1, K_2 are respectively
the concentrations of added NaOH, hydroxyl ions,
hydrogen ions and aspartic acid units, molar fraction
of II and dissociation constants (defined by concen-
trations) of carboxyls of I and II. The polymer effect
may be reduced for $\alpha < 0.7$ (MORAWETZ 1965, SAUDEK 1981)
by the presence of a neutral salt, in this work with
2 molal NaCl. A set of n experimental points is ob-
tained in the equilibrium PT: H_i, calculated from the
measured pH and the activity coefficient of H^+, and
α_i, calculated according to Eq.(2) from the added

titrant volume, pH and activity coefficient of H^+, $i=1,2,\ldots,n$. K_1, K_2 and k may be evaluated by the least squares method: A set of n equations (4) is obtained after substitution (3) and introduction of the experimental data pairs α_i and H_i in Eq.(1):

$$x = K_1K_2 , \qquad y = K_1+K_2, \qquad z = K_2k-K_1k-K_2 \qquad (3)$$

$$x(\alpha_i-1) + y\alpha_iH_i + zH_i + \alpha_iH_i^2 = \varepsilon_i \qquad (4)$$

where ε_i is residuum of the determination. The minimalization condition for the residual sum of squares $\sum\limits_{i=1}^{n} \varepsilon_i^2$ leeds to following equations:

$$x \sum_{i=1}^{n} (\alpha_i-1)^2 + y \sum_{i=1}^{n} (\alpha_i-1)H_i\alpha_i + z \sum_{i=1}^{n} H_i(\alpha_i-1) + \sum_{i=1}^{n} \alpha_iH_i^2(\alpha_i-1) = 0$$

$$x \sum_{i=1}^{n} (\alpha_i-1)H_i\alpha_i + y \sum_{i=1}^{n} H_i^2\alpha_i^2 + z \sum_{i=1}^{n} H_i^2\alpha_i + \sum_{i=1}^{n} \alpha_i^2H_i^3 = 0 \qquad (5)$$

$$x \sum_{i=1}^{n} (\alpha_i-1)H_i + y \sum_{i=1}^{n} \alpha_iH_i^2 + z \sum_{i=1}^{n} H_i^2 + \sum_{i=1}^{n} \alpha_iH_i^3 = 0$$

The solution of (5) provides x, y and z and, according to (4), K_1, K_2 and k.
The results may be further improved supposing that the set of m various poly(Asp) samples differs in k only, K_1, K_2 being identical for all polymers. The least squares method leads to (m+2) equations (7) after the substitution (6):

$$x = K_1K_2 , \qquad y = K_1+K_2, \qquad z_h = K_2k_h-K_1k_h+K_2 \qquad (6)$$

$$x \sum_{h=1}^{m} \sum_{i=1}^{n_h} (\alpha_{ih}-1)^2 + y \sum_{h=1}^{m} \sum_{i=1}^{n_h} \alpha_{ih}H_{ih}(\alpha_{ih}-1) +$$

$$+ \sum_{h=1}^{m} z_h \sum_{i=1}^{n_h} H_{ih}(\alpha_{ih}-1) + \sum_{h=1}^{m} \sum_{i=1}^{n_h} \alpha_{ih}H_{ih}^2(\alpha_{ih}-1) = 0$$

$$x \sum_{h=1}^{m} \sum_{i=1}^{n_h} (\alpha_{ih}-1)H_{ih}\alpha_{ih} + y \sum_{h=1}^{m} \sum_{i=1}^{n_h} \alpha_{ih}^2H_{ih}^2 +$$

$$+ \sum_{h=1}^{m} z_h \sum_{i=1}^{n_h} \alpha_{ih} H_{ih}^2 + \sum_{h=1}^{m} \sum_{i=1}^{n_h} \alpha_{ih}^2 H_{ih}^3 = 0 \qquad (7)$$

$$x \sum_{i=1}^{n_h} (\alpha_{ih}-1) H_{ih} + y \sum_{i=1}^{n_h} \alpha_{ih} H_{ih}^2 + z_h \sum_{i=1}^{n_h} H_{ih}^2 + \sum_{i=1}^{n_h} \alpha_{ih} H_{ih}^3 = 0$$

where $h=1,2,\ldots,m$, $i=1,2,\ldots,n_h$, k_h is the molar fraction of II in h-th poly(Asp) sample, α_{ih}, H_{ih} and n_h are respectively the i-th degree of ionization, i-th hydrogen ion concentration and number of the experimental points obtained by PT of h-th sample.

The calculations were performed on a Wang 2200 calculator.

Results and Discussion

The calculation was carried out for α_i in the interval 0.08-0.7; smaller α_i were not used because of a relatively great experimental error (the accuracy of pH determination at low values is low and small error in pH causes great error in α_i at low α_i); higher α_i were not used because the polymer effect may not be compensated enough for these values even in a 2 molal NaCl. About 40 experimental points were used from one PT experiment.

Table I presents the results obtained according to Eqs (5) (method A - each PT evaluated separately) and (7) (method B - PT of all 5 samples evaluated together). The correlation coefficient of experimental α_i and regression curve was greater than 0.99 standard deviation for α_i lower than 10^{-5} in all cases. The supposition that K_1 and K_2 do not differ for various poly(Asp) samples of different k and molecular weight is acceptable. The results of the method B are based on a higher number of experimental points and may be regarded as more pertinent. Figure 1a shows the titration curve of poly(Asp)III, Fig. 1b represents its modified titration curve. The regression curve fits the experimental points very good. The deviations are somewhat more apparent from the plot usually employed for the PT curves of polymers (Fig.1b). Even the points measured for $\alpha_i < 0.08$, not used in the evaluation, do not substantially deviate from the regression curve. The figures also indicate that the polymer effect was compensated enough for $\alpha_i < 0.7-0.8$. Similar agreement was obtained with all the other samples. Equation (1) is a good representation of the PT behaviour of poly(Asp) for $\alpha_i < 0.7-0.8$.

Table I presents for comparison the results obtained by means of NMR spectroscopy. The k values determined by PT are somewhat smaller but lie within
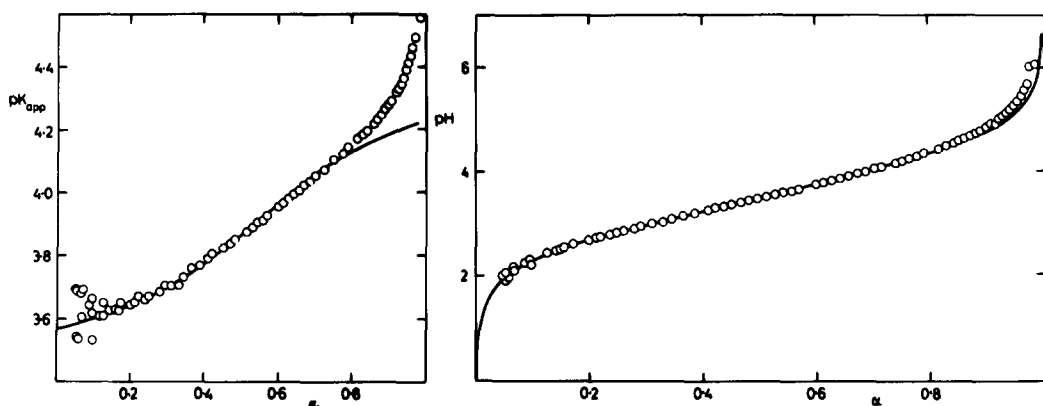
Fig.1. (a) Titration curve of poly(Asp)III. (b) Modified plot $pK_{app} = pH+(1-\alpha)/\alpha$ vs. α of the titration curve of poly(Asp)III. The dots represent experimental points, the line the regression curve.

the range of the experimental error, in good agreement with those obtained by NMR spectroscopy. PT is of course not the best method for the problem studied. Evidence of I and of II was given only indirectly by PT and assuming that the polymer effect was compensated sufficiently. NMR spectroscopy provides evidence of I and II directly. Nevertheless, the comparison in Table I shows that PT leads to reliable results.

TABLE I

Analysis of poly(Asp) samples (k - molar fraction of II, K_1, K_2 - dissociation constants of I and II, $pK_1 = -\log K_1$, $pK_2 = -\log K_2$)

Sample[a]	$[\eta]$[b] ml/g	NMR[c] k	Method A[d] pK_1	pK_2	k	Method B[e] pK_1	pK_2	k
I	29	0.80	3.37	4.60	0.75			0.76
III	44	0.56	3.30	4.48	0.50			0.50
IV	43	0.60	3.31	4.46	0.58	3.32	4.50	0.57
V	41	0.62	3.45	4.42	0.59			0.59
VII	70	0.75	3.37	4.41	0.72			0.71

[a] Preparation and characterization: PIVCOVÁet al.
[b] Intrinsic viscosity in 0.2 M NaCl pH = 7.3
[c] Evaluated from the relative intensities of the ^{13}C NMR CH_2 bands at pH = 9.2 (PIVCOVÁ et al. 1981)
[d] Evaluated according to Eqs (5)
[e] Evaluated according to Eqs (7)

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