ELSEVIER

Biophysical Chemistry 57 (1996) 133–142

Biophysical
Chemistry

# Interaction of calcium ions and polyelectrolytes

Shozo Iida

*Department of Physics, School of Science, Nagoya University, Furo-cho, Chikusa, Nagoya, Japan, 464-01*

Received 15 June 1994; revised 13 March 1995; accepted 21 April 1995

## Abstract

Interaction of $Ca^{2+}$ ions with poly(acrylic acid) (PAA) or poly(methacrylic acid) (PMA) was studied using a $Ca^{2+}$ ion sensitive electrode. When the PAA solution was neutralized with $Ca(OH)_2$, the $Ca^{2+}$ activity had a maximum around the degree of neutralization 0.5 and decreased with further increase of $Ca(OH)_2$, being similar to the behavior of the $Ca^{2+}$ activity in a maleic acid copolymer solution as reported previously. When the polymer concentration was as low as 0.1 mM, this peak in the $Ca^{2+}$ activity was not observed and the counterion condensation theory held. The decrease of the $Ca^{2+}$ activity in PAA solution at the degree of neutralization unity was depressed by the presence of several millimolar KCl. The $Ca^{2+}$ activity in the PAA solution at the low degree of neutralization was increased by the presence of dilute KCl and decreased by the presence of concentrated KCl. The decrease of the $Ca^{2+}$ activity in PMA solution was observed also at the degree of neutralization unity, but its extent was small compared with that of the PAA solution and the maximum of the $Ca^{2+}$ activity was shifted to the degree of neutralization 0.75. The effects of KCl on the $Ca^{2+}$ activity in the PMA solution were almost the same as those in the PAA solution. Interpretations of the behavior of the $Ca^{2+}$ activity were discussed.

*Keywords:* Poly(acrylic acid); Poly(methacrylic acid); Calcium; Counterion condensation; Polyelectrolytes

## 1. Introduction

Counterion condensation and the additivity rule of counterion activity are important properties of polyelectrolytes derived from simple theories [1–6]. We reported that the counterion condensation theory does not hold for a divalent counterion when a maleic acid copolymer solution is neutralized with $Ca(OH)_2$ [7]. The $Ca^{2+}$ activity had a maximum at the degree of neutralization 0.5 and decreased by increasing $Ca(OH)_2$ concentration beyond it. It is important to elucidate whether this phenomenon is general for linear poly(carboxylic acid) solution or is particular for the maleic acid copolymer solution because the maleic acid copolymers contain pairs of carboxyl groups and $Ca^{2+}$ ion may bind locally to a paired carboxyl group. We report here the interaction of the $Ca^{2+}$ ion with poly(acrylic acid) (PAA) and poly(methacrylic acid) (PMA) which are typical linear polyelectrolytes. PMA is known to make a conformational change when titrated with KOH at low pH but PAA have the randomly coiled conformation at low pH [8,9]. We aimed to investigate how the conformational change of polymer affects the activity of $Ca^{2+}$ ion.

## 2. Materials and methods

Poly(acrylic acid) and poly(methacrylic acid) were purchased from Scientific Polymer Products, Inc. (New York). The stock polymer solutions were pre-

*SSDI* 0301-4622(95)00068-2

pared by dissolving PAA or PMA in dilute KOH solutions. The solutions were dialyzed against 10 mM HCl solution and finally dialyzed against pure water. Concentration of the stock solution was determined by titrating with $Ba(OH)_2$ solution. 99.9% $Ca(OH)_2$ and $CaCl_2$ were purchased from Wako Pure Chemical Industries, Ltd. Ultra pure (Suprapur) KCl was purchased from Merck. $Ca(OH)_2$ was used in saturated solution whose contaminated carbonate was eliminated by centrifugation. Carbonate free KOH solution was prepared according to Powell and Miller [10] using Chelex 100 (Bio Rad) column. Concentration of alkaline solution was determined by titration with standard HCl solution (1.000 M: Wako Pure Chemical Industries).

The $Ca^{2+}$ activity was measured by a PHM85 pH meter using a F2112Ca $Ca^{2+}$ ion sensitive electrode (Radiometer, Copenhagen) and a handmade silver–silver chloride reference electrode as mentioned previously [11]. A small, thin pinhole with electric resistance of about 400 kΩ was used. The concentration of KCl eluted from the reference electrode was measured using a F2312K $K^+$ ion sensitive electrode (Radiometer, Copenhagen) and was found to be less than $10^{-5}$ M in the experimental condition. Asymmetric potentials were less than 0.5 mV in the presence of 1 mM KCl. Nitrogen gas saturated with water vapor was flushed continuously over the titration solution. The cell was immersed in a temperature controlled bath (25 ± 0.02°C).

The degree of neutralization, $\beta$, of the polymer solution is defined as

$$\beta = [BOH]/[Cp]$$

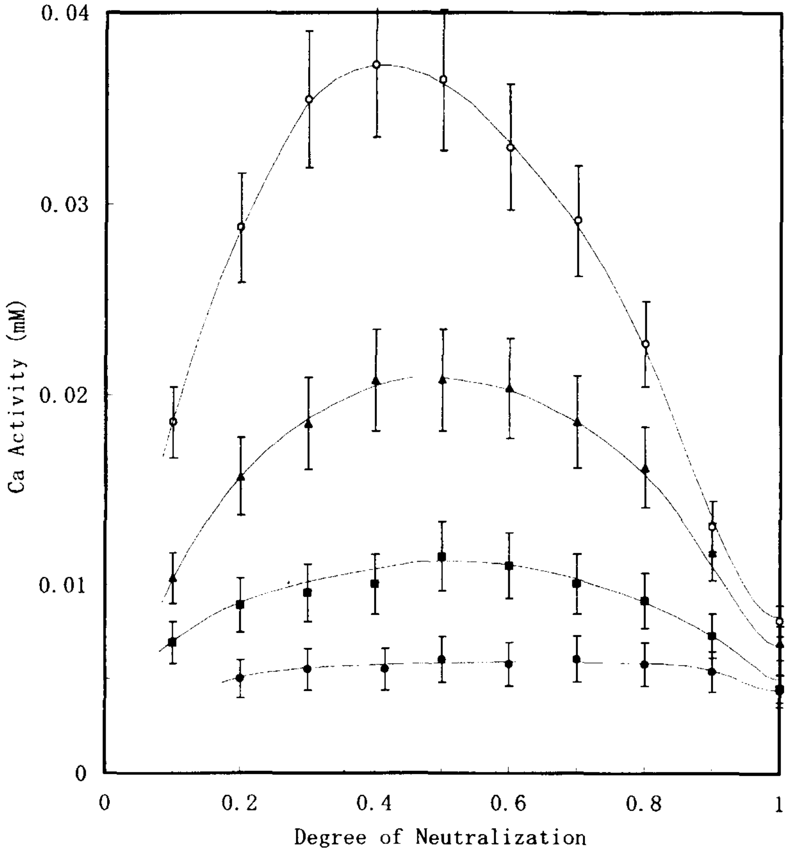

Fig. 1. $Ca^{2+}$ activity of the PAA solutions neutralized with $Ca(OH)_2$ in the absence of KCl. PAA concentrations are 0.1 (●), 0.25 (■), 0.5 (▲), and 1 mM (○).

where [BOH] is the number of normality of base added, and [Cp] is the polymer concentration in normality.

## 3. Results

### 3.1. $Ca^{2+}$ activity of PAA solution neutralized with $Ca(OH)_2$

The PAA solution was neutralized with $Ca(OH)_2$ in the absence of salt ions. The $Ca^{2+}$ activity was measured by using a $Ca^{2+}$ ion sensitive electrode. The $Ca^{2+}$ activity increased with increasing degree of neutralization when $\beta < 0.5$ and decreased beyond $\beta = 0.5$ the polymer concentrations were 0.25 and 0.5 mM as shown in Fig. 1. These results are similar to those obtained in the maleic acid copolymer [7] but the $Ca^{2+}$ activity curves had a dome-like shape and the peak of the $Ca^{2+}$ activity was not so sharp as in the maleic acid copolymer. The maximum of $Ca^{2+}$ activity was shifted from $\beta = 0.5$ to lower values at a polymer concentration higher than 1 mM. When the polymer concentration was very low (i.e. 0.1 mM), the $Ca^{2+}$ activity was constant ($A_{Ca} = 0.0058$ mM) in $0.2 \leqslant \beta \leqslant 0.9$ showing that the counterion condensation theory holds.

The $Ca^{2+}$ activity of PAA solution depended on the concentration of added salt. Presence of a few

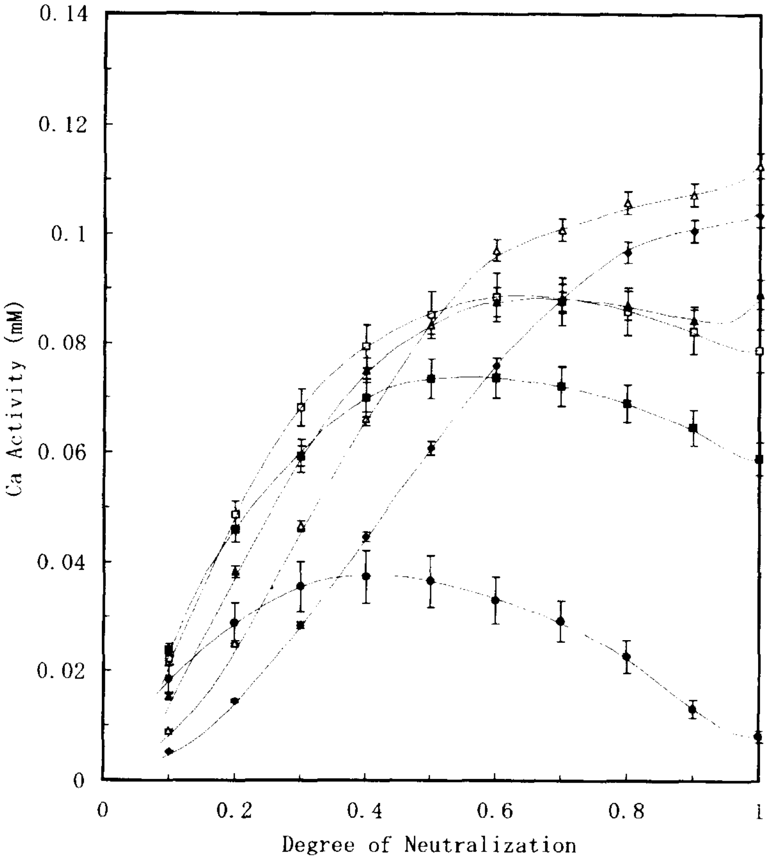

Fig. 2. KCl concentration dependence of $Ca^{2+}$ activity in the PAA solution. PAA solutions were neutralized with $Ca(OH)_2$. KCl concentrations were 0 (●), 3 (■), 10 (□), 30 (▲), 100 (△), and 300 mM (◆). The PAA concentration was 1 mM.

Table 1
$Ca^{2+}$ activity of the PAA solution at $\beta = 0.1$ and $\beta = 1$

| | $Ca^{2+}$ activity (mM) | |
|---|---|---|
| [KCl] (mM) | $\beta = 0.1$ | $\beta = 1.0$ |
| 0 | 0.0185 ± 0.0024 | 0.0081 ± 0.0015 |
| 1 | 0.0222 ± 0.0016 | 0.0255 ± 0.0018 |
| 3 | 0.0238 ± 0.0012 | 0.0590 ± 0.0031 |
| 10 | 0.0222 ± 0.0011 | 0.0786 ± 0.0040 |
| 30 | 0.0154 ± 0.0005 | 0.0890 ± 0.0024 |
| 100 | 0.0090 ± 0.0002 | 0.1126 ± 0.0024 |
| 300 | 0.0053 ± 0.0001 | 0.1034 ± 0.0022 |

PAA solutions were neutralized with $Ca(OH)_2$ in the absence of KCl. The PAA concentration was 1 mM.

millimolar KCl increased the $Ca^{2+}$ activity when neutralized with $Ca(OH)_2$ as shown in Fig. 2. The $Ca^{2+}$ activity at the degree of neutralization of unity is listed in Table 1. It increased greatly by addition of a small amount of KCl. It increased by 3 times in 1 mM KCl or 10 times in 10 mM KCl, compared with that in the absence of KCl. The peak of $Ca^{2+}$ activity which was observed clearly at $\beta = 0.4$ at a polymer concentration of 1 mM in the absence of the salt was not observed in the presence of more than 100 mM KCl.

The $Ca^{2+}$ activity at $\beta = 0.1$ increased with increasing KCl concentration below 3 mM and decreased with further increase of KCl beyond 3 mM as shown in Table 1. The increase of the $Ca^{2+}$ activity in the range of low concentration of KCl was not so drastic at $\beta = 0.1$ as that at $\beta = 1$. The decrease of the $Ca^{2+}$ activity at $\beta = 0.1$ with increasing KCl concentration was dominant above 30 mM (Table 1).

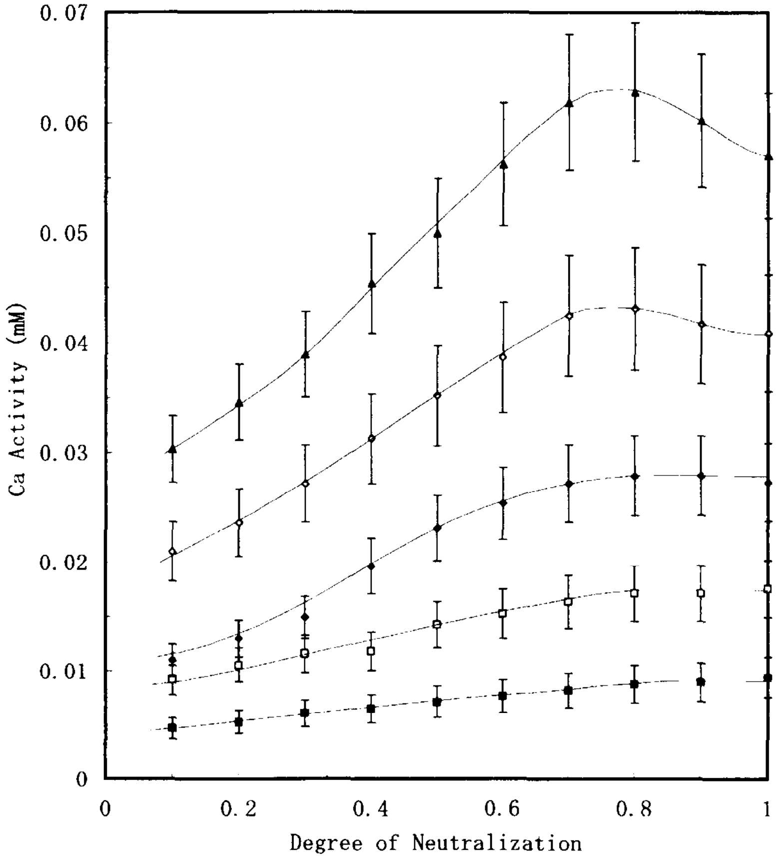

Fig. 3. $Ca^{2+}$ activity of the PMA solutions neutralized with $Ca(OH)_2$. PMA concentrations were 0.1 (■), 0.25 (□), 0.5 (◆), 1 (◇), and 1.5 (▲) mM.

### 3.2. $Ca^{2+}$ activity of PMA solution neutralized with $Ca(OH)_2$

The $Ca^{2+}$ activity of PMA solution neutralized with $Ca(OH)_2$ differed from that of PAA solution. When the polymer concentration was higher than 1 mM, the $Ca^{2+}$ activity was increased by increasing $Ca(OH)_2$ concentration until $\beta = 0.75$, and decreased beyond it as shown in Fig. 3. However, the extent of the decrease of the $Ca^{2+}$ activity at the degree of neutralization unity was smaller than that of the PAA solution. The $Ca^{2+}$ activity in the PAA solution at the degree of neutralization unity was 25% of the maximum value when the polymer concentration was 1 mM, whereas it was 95% of the maximum value for PMA.

The $Ca^{2+}$ activity increased monotonically by increasing the $Ca(OH)_2$ concentration when the polymer concentration was less than 0.5 mM (Fig. 3). The $Ca^{2+}$ activity was not constant in $0.2 \leqslant \beta \leqslant 0.9$ even when the polymer concentration was 0.1 mM and the counterion condensation theory did not hold in the PMA solutions, in contrast with the case of the PAA solutions.

The $Ca^{2+}$ activity of the PMA solution also depended on the added KCl concentration. The peak of the $Ca^{2+}$ activity was not observed when the KCl concentration was higher than 10 mM (Fig. 4). Addition of KCl up to 10 mM increased the $Ca^{2+}$ activity for all degrees of neutralization. The $Ca^{2+}$ activity at the degree of neutralization 0.1 and unity are listed in Table 2. The $Ca^{2+}$ activity at the degree of neutralization unity increased by 3 times when adding 10 mM KCl compared to that in the absence of KCl.

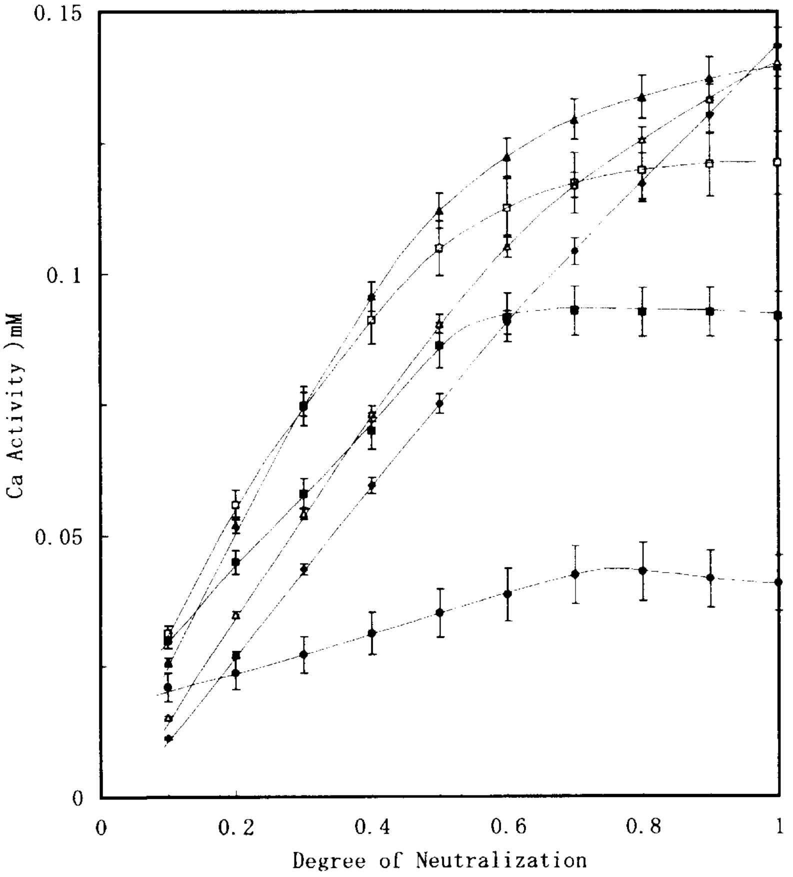

Fig. 4. KCl concentration dependence of $Ca^{2+}$ activity in the PMA solution. PMA solutions were titrated with $Ca(OH)_2$. KCl concentrations were 0 (●), 3 (■), 10 (□), 30 (▲), 100 (△), and 300 mM (◆). The PMA concentration was 1 mM.

Table 2
$Ca^{2+}$ activity of the PMA solution

| [KCl] mM | $Ca^{2+}$ activity (mM) | |
|---|---|---|
| | $\beta = 0.1$ | $\beta = 1.0$ |
| 0 | 0.0209 ± 0.0027 | 0.0408 ± 0.0058 |
| 1 | 0.0297 ± 0.0021 | 0.0783 ± 0.0060 |
| 3 | 0.0299 ± 0.0015 | 0.0918 ± 0.0051 |
| 10 | 0.0313 ± 0.0016 | 0.1212 ± 0.0067 |
| 30 | 0.0258 ± 0.0008 | 0.1395 ± 0.0045 |
| 100 | 0.0152 ± 0.0003 | 0.1404 ± 0.0031 |
| 300 | 0.0111 ± 0.0002 | 0.1433 ± 0.0032 |

PMA solutions were neutralized with $Ca(OH)_2$ in the absence of KCl. The PMA concentration was 1 mM.

The increase of $Ca^{2+}$ activity by the addition of KCl at the degree of neutralization unity was less than that of the PAA solution. It increased with increasing KCl concentration up to 300 mM. The $Ca^{2+}$ activity at $\beta = 0.1$ increased with increasing KCl concentration up to 10 mM and decreased beyond it (Table 2).

### 3.3. $Ca^{2+}$ activity neutralized with KOH

The PAA solution was neutralized with KOH in the presence of 0.1, 0.2, 0.3, 0.4, and 0.5 mM $CaCl_2$. The concentration of PAA was 1 mM, and the $Ca^{2+}$ concentration of 0.5 mM was to saturate the carboxyl groups of the polymer. Measurements were done in the presence of 1 mM or 300 mM KCl. The condition of 1 mM KCl was employed because the increase of the KCl concentration by adding KOH may bring about a large $Ca^{2+}$ activity change in the absence of KCl as shown in Fig. 2 (also see Table 1). The $Ca^{2+}$ activity neutralized with KOH in the

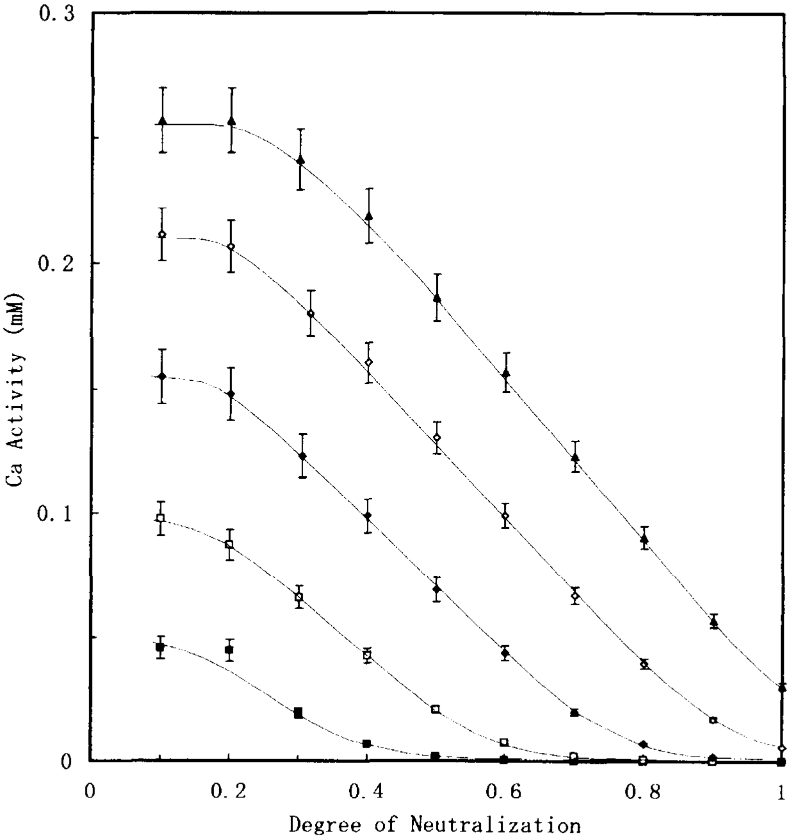

Fig. 5. (A) $Ca^{2+}$ activity of the PAA solution neutralized with KOH in the presence of 1 mM KCl. PAA concentration was 1 mM. $CaCl_2$ concentrations were 0.1 (■), 0.2 (□), 0.3 (◆), 0.4 (◇), and 0.5 mM (▲). (B) $Ca^{2+}$ activity of the PMA solution neutralized with KOH in the presence of 1 mM KCl. PMA concentration was 1 mM. Symbols are same as in (A).

presence of 1 mM KCl was shown in Fig. 5A.

The $Ca^{2+}$ activity did not change up to $\beta = 0.2$ and decreased linearly beyond it by increasing KOH concentration, irrespective of the added $CaCl_2$ concentration. The charge on the polymer molecule should increase linearly by increasing the degree of ionization until $\xi = e_0^2/\epsilon kTd$ reaches to 1/2 and then it is constant beyond that degree of ionization following the counterion condensation theory, where $e_0$, $\epsilon$, and $d$ is proton charge, dielectric constant of the solvent, and the distance between charges on the polymer, respectively [1,3,7]. Because the value of the degree of ionization at $\xi = 1/2$ is 0.18 for fully extended conformation of PAA, the experimental results shown in Fig. 5A show that the counterion condensation theory holds in these experiments: $Ca^{2+}$ was not bound up to $\beta = 0.2$ but bound to keep the apparent charge density on the polymer molecule constant beyond it. The $Ca^{2+}$ activity of PMA solution neutralized with KOH in the presence of $CaCl_2$ was shown in Fig. 5B. The $Ca^{2+}$ activity decreased linearly by increasing the degree of neutralization even in $0.1 \leqslant \beta$, in contrast with the results of PAA solution.

The behavior of the $Ca^{2+}$ activity in 300 mM KCl differed from that in 1 mM KCl. In the presence of 300 mM KCl, the $Ca^{2+}$ activity of PAA solutions neutralized with KOH was increased by increasing the degree of neutralization up to 0.5 and then decreased as shown in Fig. 6. An increase of the $Ca^{2+}$ activity was observed for $\beta < 0.5$ in contrast with the increase of the electric charge on the poly-

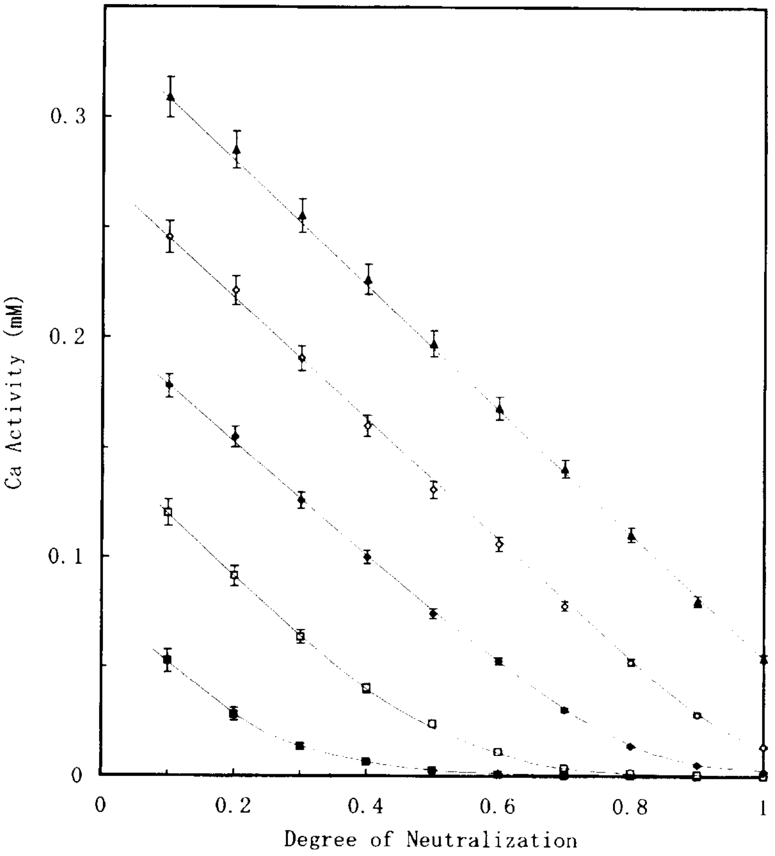

Fig. 5 (continued).

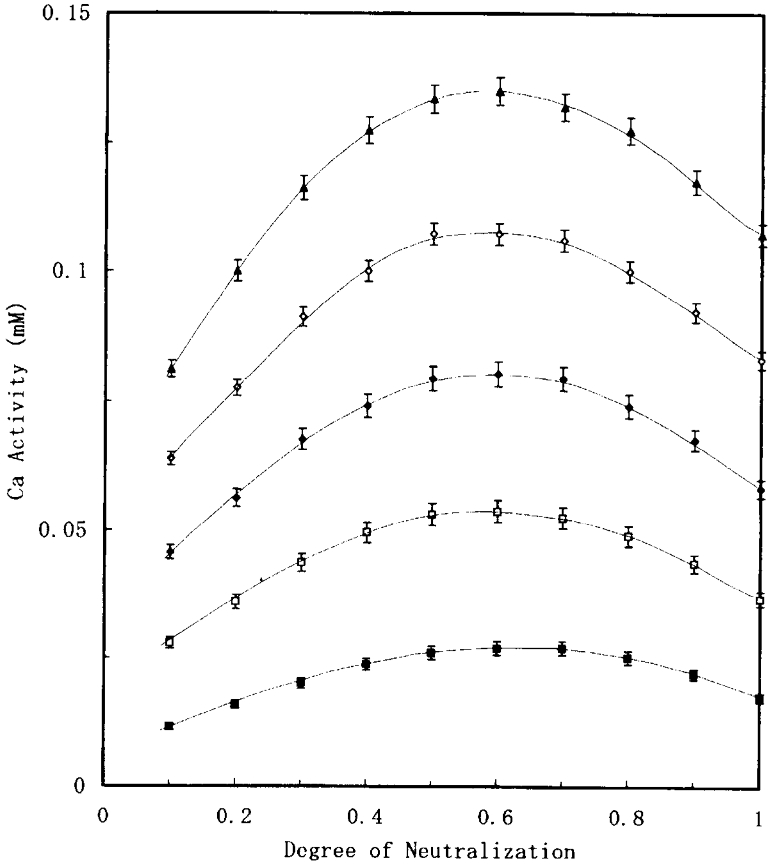

Fig. 6. $Ca^{2+}$ activity of the PAA solution neutralized with KOH in the presence of 300 mM KCl. PAA concentration was 1 mM. $CaCl_2$ concentrations were 0.1 (■), 0.2 (□), 0.3 (♦), 0.4 (◇), and 0.5 mM (▲).

mer molecules by neutralization with KOH. This increase of the $Ca^{2+}$ activity was observed also in the PMA solution (data not shown).

## 4. Discussion

We reported previously that the $Ca^{2+}$ activity had a peak at the degree of neutralization 0.5 when the maleic acid copolymer solution was neutralized with $Ca(OH)_2$ [7]. It is important to confirm whether this phenomenon is general for linear poly(carboxylic acid) or specific for the maleic acid copolymer because the maleic acid copolymer contains pairs of carboxyl groups. In this work we have found that the $Ca^{2+}$ activity in the PAA solution has a peak when it is neutralized with $Ca(OH)_2$ as shown in Fig. 1, suggesting that this phenomenon is general for linear poly(carboxylic acid).

We discussed previously a model assuming local strong binding of $Ca^{2+}$ to a pair of carboxyl groups of the maleic acid copolymer [7]. In the present case, however, presence of salt ions at low concentrations in the PAA solution affected the $Ca^{2+}$ activity of the solution. The $Ca^{2+}$ activity neutralized with $Ca(OH)_2$ at the degree of neutralization unity increased by 3 times in 1 mM KCl and by 7 times in 3 mM, as compared with that in the absence of KCl as shown in Table 1. The decrease of $Ca^{2+}$ activity at the degree of neutralization unity was depressed by the presence of even a few millimolar KCl and the peak of the $Ca^{2+}$ activity was not observed in 100 mM KCl. Presence of salt ions screens the electric field around the macro-ion. Debye length, $\lambda_d$, is a parameter to characterize the distance where the electric potential is screened by salt ion. $\lambda_d$ is defined as $\lambda_d = 1/\kappa$, where $\kappa$ is Debye–Hückel parameter [12]. Values of $\lambda_d$ for 1 mM, 3 mM, and 10 mM KCl were 9.7 nm, 5.6 nm and 3.1 nm, respectively. On the other hand, the $Ca^{2+}$ activity in solutions of the polymer concentration of 1 mM at $\beta = 1$ were 0.0081, 0.0255, 0.059, and 0.0786 mM and the activity coefficients were 0.016, 0.051, 0.118, and 0.157 in the presence of 0, 1, 3, and 10 mM KCl, respectively. The activity coefficient of 0.016 shows that almost all of the $Ca^{2+}$ ions were bound on the macro-ion and only 2% of the total $Ca^{2+}$ ions are free from the macro-ion. A number of $Ca^{2+}$ ions were released from the macro-ion by adding a few millimolar KCl. The value of $\lambda_d$ of 5.6 nm, or 3 mM KCl, for example, is larger than the distance between the adjacent carboxyl groups of the macro-ion, and it is unlikely that the release of the $Ca^{2+}$ by KCl was due to screening of local ionic binding of $Ca^{2+}$ ion to adjacent carboxyl. If the decrease of the $Ca^{2+}$ activity at the degree of neutralization of unity is due to strong binding to a paired carboxyl group, the pair may not be formed with the adjacent carboxyl groups but formed with the groups at a distance along the chains or those of the other molecules. It is possible that the conformation of the polymer molecule is changed by the pairing of the carboxyl groups or the inter-molecular conformation is changed by the pairing. The polymer assembly mentioned previously [7] is possibly one of the last case. Anyway, the experimental results suggest that the screening of electrostatic interaction between distant carboxyl groups brings about the increase of the $Ca^{2+}$ activity at the degree of neutralization of unity. It is necessary to study intra- and inter-conformation of the polymer in the presence of $Ca^{2+}$ ions.

The behavior of the $Ca^{2+}$ activity in the PMA solution was different from that in the PAA solution. The $Ca^{2+}$ activity of the PMA solution at the degree of neutralization unity in the absence or the presence of small amounts of KCl was remarkably larger than that of PAA solution as shown in Tables 1 and 2. The behavior of the $Ca^{2+}$ activity in PAA and in PMA solution by neutralization with KOH in the presence of $CaCl_2$ also differed in the range of $0.1 \leqslant \beta \leqslant 0.2$ as shown in Fig. 5A and B. One possible interpretation of the differences in the $Ca^{2+}$ activity between PAA and PMA solutions is that it is due to the difference in the conformation of PAA and PMA molecules in the solutions. It was reported that PMA molecules take a compact conformation at low pH values when the solution is titrated with KOH [8,9]. The PMA molecule neutralized with $Ca(OH)_2$ may take a similar conformation as those neutralized with KOH at low pH and maintain the conformation in the region. On the other hand the PAA molecule may be extended by increasing the degree of neutralization at a low pH. As a result, the ratio of the charge density of PMA molecule at $\beta = 0.2$ to $\beta = 0.1$ may be higher than that of PAA molecules and the ratio of the activity coefficient of $Ca^{2+}$ of PMA solution at $\beta = 0.2$ to $\beta = 0.1$ is lower than that of the PAA solution. The activity coefficient of $Ca^{2+}$ in 1 mM PMA solutions neutralized with $Ca(OH)_2$ in the absence of KCl was 0.42 and 0.24 for $\beta = 0.1$ and 0.2. On the other hand the activity coefficient of $Ca^{2+}$ in a 1 mM PAA solution in the same conditions were 0.37 and 0.29, respectively. These results are consistent with the idea that the compact conformation of PMA is maintained at low pH. The difference of the $Ca^{2+}$ activity in PAA and in PMA solutions at the degree of neutralization unity may be due to the difference of conformation at the degree of neutralization unity. We need further study about the conformation of PAA and PMA molecules neutralized with $Ca(OH)_2$.

The $Ca^{2+}$ activity of the PAA solution in the presence of 300 mM KCl neutralized with KOH was increased by increasing the degree of neutralization for $0.1 \leqslant \beta \leqslant 0.5$ as shown in Fig. 6. A similar increase of the $Ca^{2+}$ activity in the presence of 300 mM KCl was observed in the PMA solution. This increase in the $Ca^{2+}$ activity is opposed to the increase of the charge density on the polymer. The increase of the $Ca^{2+}$ activity by adding KOH was observed only in the presence of a high concentration KCl. A high concentration of KCl reduces largely the electrostatic interaction between the $Ca^{2+}$ ion and the macro-ion, and some kind of short range interaction may play an essential role in this phenomenon.

## Acknowledgements

We thanks Professors F. Oosawa, A. Minakata, and H. Kijima for helpful discussion and suggestions.

## References

[1] F. Oosawa, Polyelectrolytes, Dekker, New York, 1971.

[2] F. Oosawa, Biopolymers, 6 (1968) 135-144.

[3] G.S. Manning, Q. Rev. Biophys., 11 (1978) 179-246.

[4] G.S. Manning, J. Chem. Phys., 51 (1969) 924-933.

[5] G.S. Manning, J. Chem. Phys., 51 (1969) 3249-3252.

[6] P.J. Heath and J.M. Schurr, Macromolecules, 25 (1992) 4149-4159.

[7] S. Iida, Biophys. Chem., 53 (1995) 219-225

[8] J.C. Leyte and M. Mandel, J. Polym. Sci., 2 (1964) 1879-1391.

[9] B. Bednář, J. Trnĕná, P. Svoboda, Š. Vajda, V. Fidler, and K. Procházka, Macromolecules, 24 (1991) 2054-2059

[10] J. Powell and M. Miller, J. Chem. Educ., 34 (1957) 330-331.

[11] S. Iida, J. Biochem., 103 (1988) 482-486.

[12] S.A. Rice and M. Nagasawa, Polyelectrolyte Solutions, Academic Press Inc., New York, 1961, pp. 81-129.