

Full Paper

Voltammetric Study of Poly Aspartic Acid-Cd²⁺ and/or Zn²⁺ Complex in the Helix-coil Transition pH Region

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Abstract- Helix-coil transition of poly aspartic acid (PASP) was investigated by dc polarography in the presence of Cd²⁺ and/or Zn²⁺ as a detecting marker which attached to the polymer. The diffusion currents (i_d) of Cd²⁺ and Zn²⁺ decreased sharply in the pH range of 3.5-7.0 due to the formation of the metal ions-PASP macromolecular complexes. The complex formation revealed on an increase in the magnitude at 222 nm of CD spectrum of the polymer, indicating that PASP forms helix structure by coordination of both ions in the corresponding pH region. The helix content of PASP, estimated by polarography is in good agreement with that by CD in neutral pH region. The degree of helicity induced by either of these two ions also can be determined independently by polarography. The size of the complex was calculated by Ilkovic's equation and depends on strongly mixing ratio of PASP residue to cation marker. On the other hand in the lower acidic pH region, the coordination mode of these ions to PASP is different from that at neutral pH region. The decreases in i_d of the ions are independent of the further formation of helix structure. The metal ions coordinate with sparsely dissociated carboxylate groups of helical part of PASP, which bring about an aggregation of polypeptide strands. The diffusion current of the metal ion attached to the polymer, therefore, is a parameter sensitive to a conformational change of PASP from acidic through neutral pH region.

Keywords- Cadmium, Helix-coil transition, Polarography, Poly aspartic acid, Zinc

1. INTRODUCTION

Helix-coil transition of poly amino acids plays an important role in biophysical functions of proteins such as the protein folding-unfolding mechanism [1-3]. The amounts of helix structure and their relative configuration relates to their specific functions or tertiary structure of proteins in conjunction with β -sheet or random coil state. It is, therefore, important to study this transition for understanding a mode of action of proteins. Synthetic water-soluble homo-poly amino acid such as poly α -glutamic acid (PGA) or Poly L-Lysine (PLL) is often used as a simplest model component of natural protein to investigate not only the equilibrium state but also the dynamics of the transition of proteins by using many spectroscopic techniques, such as UV, NMR, or CD method [4-10].

Among those methods, CD is a primarily tool to study the secondary structure of many proteins and poly peptides, since the conformational change of those is sharply reflected on CD spectrum in the polypeptide backbone region of 360~180nm [8,9]. PGA is known to show a typical CD spectral pattern for helix structure or random coil state associated with this transition [8-10]. Although poly aspartic acid (PASP), like PGA, has an ionizable carboxylate side chain group and also undergoes the helix-coil transition by the presence/absence of metal ion or the change of pH or temperature, the change of CD spectral pattern by the transition of PASP is not so conspicuous as those of PGA [11,12]. PASP is likely to be shielded by the environmental ions (H^+ , OH^-) not to attain a high level of helix structure, because of shorter length of side chain [13,14]. The conformational transition of PASP is, therefore, difficult to be elucidated by CD spectrum only. So far, not so many studies have been reported on PASP compared to those for PGA, partly because of those reasons [11,12]. From electro-chemical point of view, amino acid-metal complex formation including helix-coil transition of PGA or PASP is one of the most interesting targets for an application of polarography, since those amino acids or poly amino acids have i) intrinsic side chain groups reducible at a dropping mercury electrode or ii) groups to which some reducible metal ions attached extrinsically as a marker. Moreover specific information on the complexes arose from different groups or marker ions can be independently obtained at each different reduction potential even though CD spectrum gives just the whole information of those amino acids or poly amino acids. In the previous papers, we reported that marker's i_d is a parameter sensitive to the conformational change of PGA and also provides quantitative information on the polypeptide-metal ion complex [15-17]. In this paper, we employed voltammetric method to obtain electro-chemical perspectives to the helix-coil transition of PASP-metal ion complex using cadmium ion and/or zinc ion as a marker.

2. EXPERIMENTAL

2.1. Materials

Sodium poly α -L-aspartic acid (PASP) ($M_w=35600$) was purchased from Sigma Co. Stock solutions of Cd^{2+} , Zn^{2+} and Pb^{2+} were prepared from $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$, ZnSO_4 , and $\text{Pb}(\text{CH}_3\text{COO})_2$ (Wako Pure Chemical Co. Ltd), respectively. pH of solutions was adjusted with 0.10 mol dm^{-3} HCl or 0.10 mol dm^{-3} NaOH. The ratio of a concentration of Cd^{2+} to PASP was defined as f ($=[\text{PASP}]/[\text{Cd}^{2+}]$). The value of f was controlled by adding a certain amount of PASP (molar residue of concentration) into solution of a constant concentration of metal ion in all measurements. Regents grade of sodium perchlorate was used as a supporting electrolyte at a concentration of 0.05 mol dm^{-3} for voltammetric measurements.

Cd^{2+} and Zn^{2+} were employed as a depolarizer to give a well-defined reduction wave in polarography.

2.2. Apparatus

Voltammetric measurements were carried out using a P-1100 Polarographic Analyzer (Yanaco Co. Ltd. Jpn.) with a saturated calomel electrode as a reference one. The hanging mercury dropping electrode had the following characteristics; $m=0.863 \text{ mg/s}$; $t=1.26 \text{ s}$ (forced drop time) in water with a mercury column height of 70 cm. The solutions were de-aerated for 5 min with pure nitrogen gas and left for 1 min before measurements were taken. CD measurements were carried out with a JASCO J-500A Spectropolarimeter (Japan Spectroscopic Co. Ltd).

2.3. Effect of Hg column height on the limiting current

The effect of the mercury column height on the limiting currents for both helical (pH 4.0) and random coil (pH 7.0) PASP solutions containing Cd^{2+} , Zn^{2+} was examined as a function of square root of Hg column height. The reduction current for the above ions in the presence of PASP indicates that the limiting currents for the ion were entirely diffusion controlled at a concentration of 0.05 mol dm^{-3} NaClO_4 .

2.4. Electro capillary curves in the presence of PASP

Electro-capillary curves of 0.05 mol dm^{-3} NaClO_4 containing various amounts of PASP were studied. The curves of the solutions with and without PASP were almost the same in the potential range of 0 through -1.40 V vs. SCE indicating that the presence of PASP does not affect the electro-reduction process of metal ions.

3. RESULTS AND DISCUSSION

3.1. Polarographic and CD spectra for PASP- Cd^{2+} and Zn^{2+} solutions

Fig. 1 shows dc polarograms of Cd^{2+} and Zn^{2+} solutions in the presence or absence of PASP. The reduction wave with a half wave potential of -0.59 V vs. SCE is due to the reduction of Cd^{2+} and that of -1.04 V is due to that of Zn^{2+} . In the presence of PASP ($f=6.26$), as pH increases from 3.5 to 6.9, the diffusion current (i_d) for each ion decreases steadily and reaches to an almost constant value.

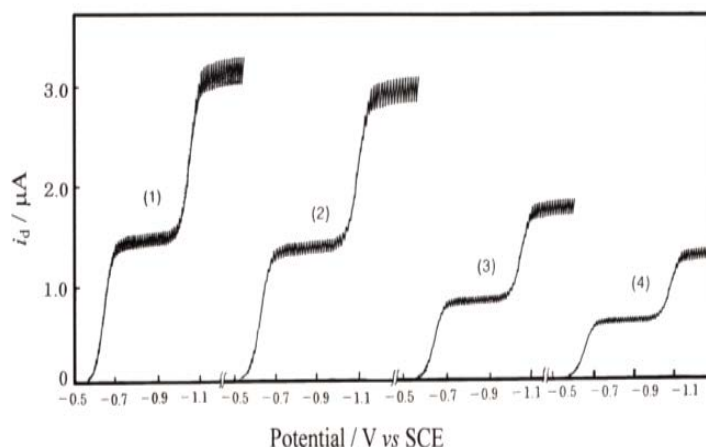


Fig. 1. The pH dependence of polarograms for PASP- Cd^{2+} and Zn^{2+} solutions. $[\text{Cd}^{2+}] = [\text{Zn}^{2+}] = 5.0 \times 10^{-4} \text{ mol dm}^{-3}$. (1) $\text{pH} = 3.60$, $f = 0$, (2) $\text{pH} = 3.60$, $f = 6.26$, (3) $\text{pH} = 4.56$, $f = 6.26$, (4) $\text{pH} = 5.57$, $f = 6.26$

Figs. 2a and 2b show CD spectra of the above solutions. In solutions with neutral or alkaline pH region PASP is known to be in a random coil state and have a CD spectrum with a weak broad negative band at 230nm and a very strong negative band at 190nm. In an acidic solution, although PASP tends to form the helix structure, its CD spectrums are not a complete helix pattern like PGA but a mixture of coil and helix ones because of its low ability of helix formation. The spectrum, therefore, consists of a very strong negative band at 195 nm and a negative broad trough around at 222 nm which evaluates the helix content [8,9]. As Fig. 2a shows the magnitude of molecular ellipticity at 222 nm ($[\theta]_{222\text{nm}}$) corresponding to the helix content decreases with pH value. On the other hand, as Fig. 2b shows, the CD spectra of PASP in the presence of Cd^{2+} and Zn^{2+} showed a partial helix pattern and are essentially the same as those of PASP obtained without any metal ion in an acidic pH reign. This result means that Cd^{2+} and Zn^{2+} play an important role in the formation of helix structure in the neutral pH region.

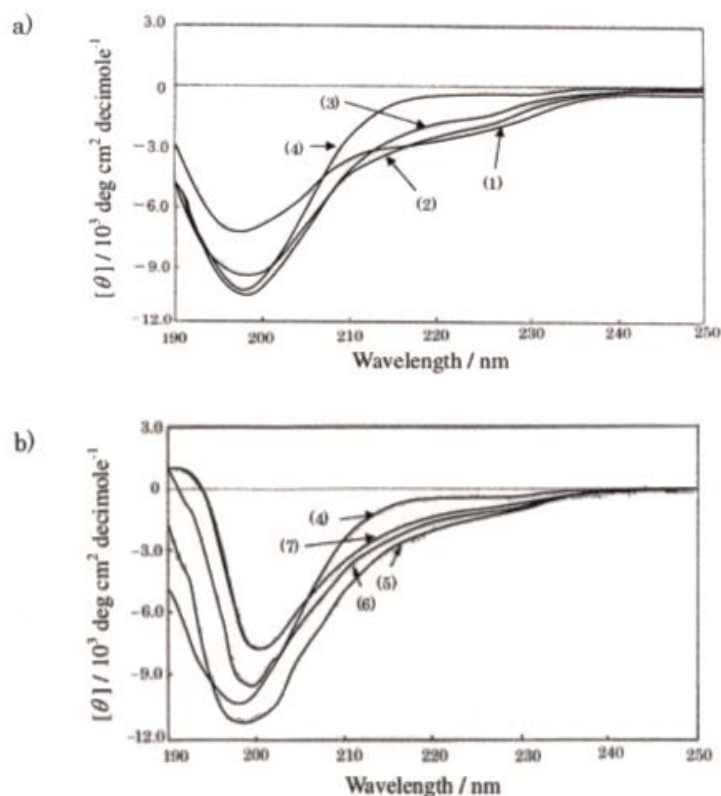


Fig. 2. (a) pH dependence of CD spectra for PASP solutions. (1) pH=3.60, (2) pH=4.50, (3) pH=5.35, (4) pH=7.00. (b) The CD spectra for PASP - Cd^{2+} and Zn^{2+} solutions at pH=7.0. (4) $f=0$, (5) $f=4.17$, (6) $f=6.26$, (7) $f=8.34$

3.2. Polarographic diffusion currents for PASP- Cd^{2+} and Zn^{2+} solutions at various ratios of f

Figs. 3-a and 3-b show that relationships between decomposed diffusion current (i_d) from the mixed polarograms of Cd^{2+} plus Zn^{2+} and pH at various value of f . In the absence of PASP, i_d for both ions remains unchanged in the pH range from *ca.* 3.0 through 7.5. Above pH 7.5, i_d decreases sharply due to the precipitation of $\text{Cd}(\text{OH})_2$ or $\text{Zn}(\text{OH})_2$ [18]. On the contrary, in the presence of PASP, i_d for both ions at various values of f , decreases gradually from pH *ca.* 3.5. The current decreases with f especially in the neutral pH region. This polarographic behavior means that both Cd^{2+} and Zn^{2+} bound to PASP to form helix structure, rendering the diffusion co-efficient of both ions much smaller. We also checked additionally prepared solutions containing $5 \times 10^{-4} \text{ mol dm}^{-3}$ Cd^{2+} or Zn^{2+} with various amounts of PASP. The decrease in i_d of each ion for those solutions with an increase in pH is the same results as obtained from the mixed polarograms of Cd^{2+} and Zn^{2+} . This similarity in polarographic results indicates that PASP forms complexes independently with Cd^{2+} and Zn^{2+} to induce the helix structure and that there is no difference in the selectivity for both ions in a formation of the helix structure.

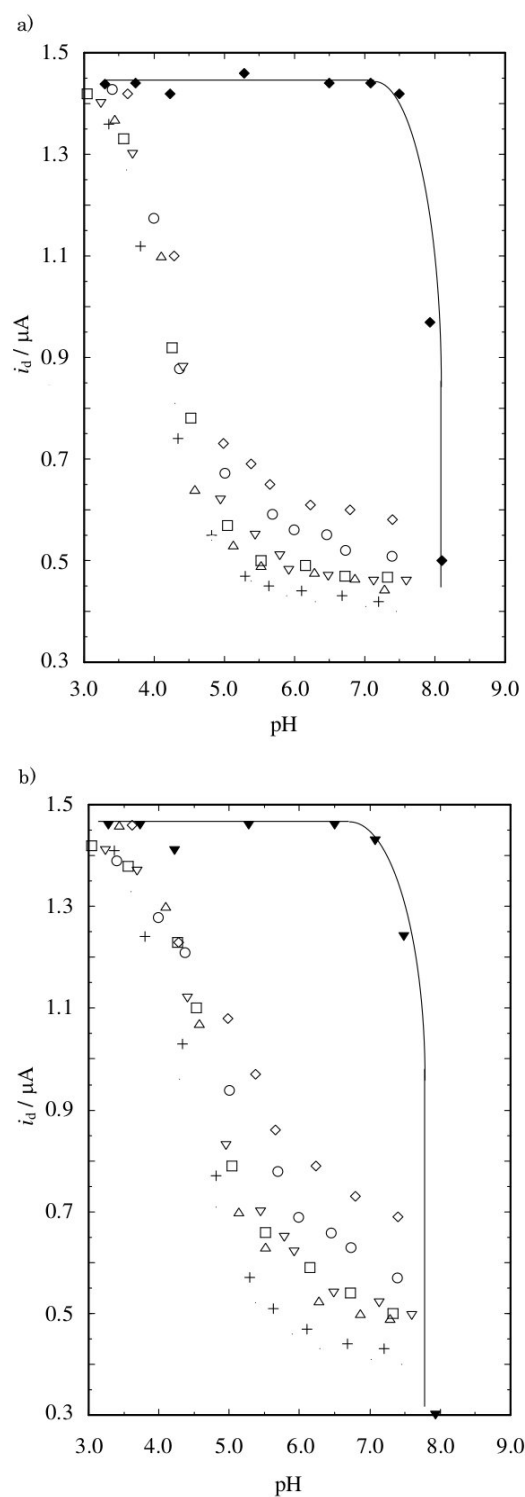


Fig. 3. (a) The relationship between the decomposed diffusion current (i_d) for Cd^{2+} in PASP- Cd^{2+} and Zn^{2+} solutions and pH at various values of f . \blacklozenge , $f=0$, \diamond , $f=3.13$, \circ , $f=4.17$, ∇ , $f=5.21$, \square , $f=6.26$, \triangle , $f=8.34$, $+$, $f=10.4$. (b) The relationship between the decomposed diffusion current (i_d) for Zn^{2+} in PASP- Cd^{2+} and Zn^{2+} solutions and pH at various values of f . \blacktriangledown , $f=0$, \diamond , $f=3.13$, \circ , $f=4.17$, ∇ , $f=5.21$, \square , $f=6.26$, \triangle , $f=8.34$, $+$, $f=10.4$.

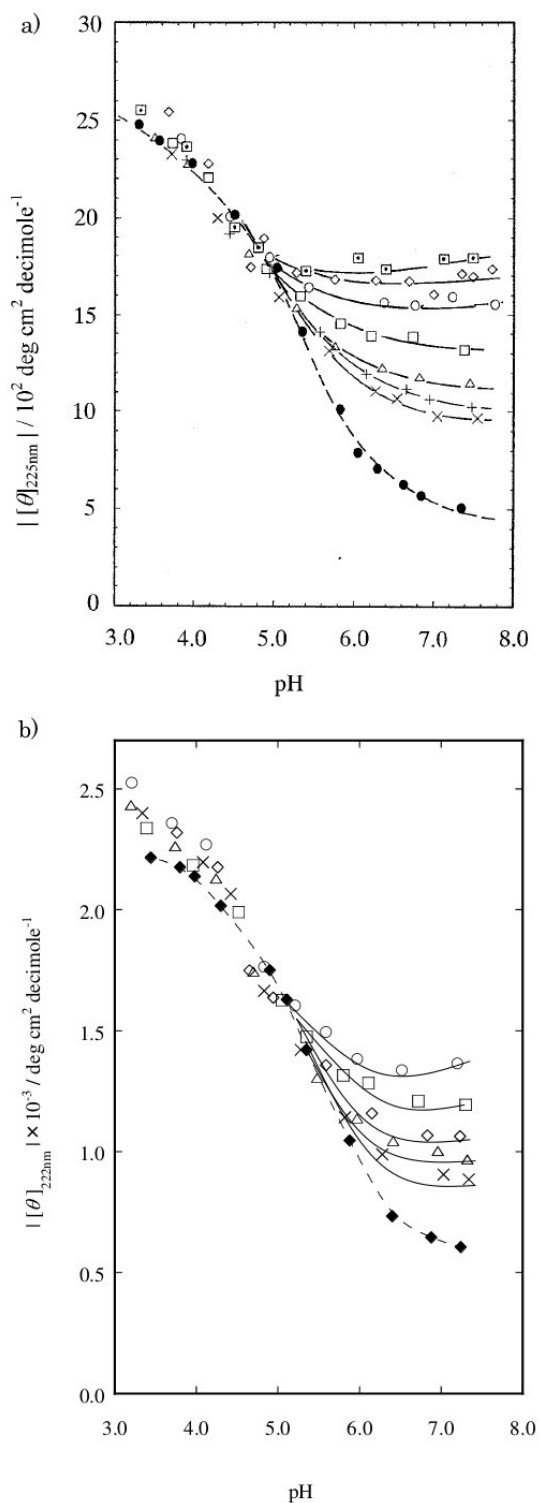


Fig. 4. (a) The relationship between the molecular ellipticity at 225nm for PASP- Cd^{2+} solutions and pH at various values of f . \bullet ; without Cd^{2+} , \square ; $f=2.09$, \diamond , $f=3.13$, \circ ; $f=4.17$, \square ; $f=6.26$, \triangle ; $f=8.34$, $+$; $f=10.4$, \times ; $f=12.5$. (b) The relationship between the magnitude of $[\theta]_{222\text{nm}}$ for PASP- Zn^{2+} solutions and pH at various values of f . \blacklozenge , $f=0$, \circ ; $f=4.17$, \square ; $f=6.26$, \diamond ; $f=8.34$, \triangle ; $f=10.4$, \times ; $f=12.5$

3.3. Molecular ellipticity at 222 nm for PASP-Cd²⁺ and Zn²⁺ solutions

The effect of pH on the magnitude of molecular ellipticity at 222 nm ($|\theta|_{222\text{nm}}$) is shown in Figs. 4-a and 4-b. The magnitude of $|\theta|_{222\text{nm}}$ is regarded as an indicator of the helix content in PASP. In acidic pH region, the ionization of carboxylate groups in PASP is almost suppressed to maintain the helix structure by hydrogen bonding between NH and CO groups in the polypeptide chain. As pH increases, carboxylate groups begin to dissociate protons in the carboxylate site, rendering PASP from helix to the random state. This transition is quantitatively explained by tracing the dashed curve from acidic to neutral pH region in Figs. 4-a and b. The helix content in PASP (without Cd²⁺ or Zn²⁺ (dashed curve)) decreases linearly with an increase in pH from 3.5 to 5.0 whether Cd²⁺ or Zn²⁺ is present or not. In this pH region, therefore, both ions do not contribute to the helix formation. From CD spectroscopic point of view, both ions just only present or attach to carboxylate groups without any contribution to the helix formation, although i_d for both ions showed a decrease tendency in this pH region. Beyond pH 5.0, the magnitude of $|\theta|_{222\text{nm}}$ decreases further to a value which shows 0% helix structure at pH 7.8. When Cd²⁺ or Zn²⁺ presents, however, beyond pH 5.0 the role of protons of carboxylate on the formation helix structure is gradually replaced by Cd²⁺, Zn²⁺ so as to suppress the ionized groups. Thus, the deviation of those curves for Cd²⁺-PASP or Zn²⁺-PASP solutions from dashed curve (PASP only) in the pH region of 5.0-7.0 begin to be observed. The degree of deviation of those curves from the dashed one becomes large with an increase in the ratio of Cd²⁺, Zn²⁺ per [PASP] residue. In this pH region (*ca.* 5.0-below 7.0), the helix formation is sustained not only by H⁺ but also by Cd²⁺, Zn²⁺ which is randomly distributed on the dissociated carboxylate groups. Above pH 7.0 PASP forms the helix structure not by H⁺ but by Cd²⁺, Zn²⁺ which directly correlates with the decrease in i_d . As pH increases from 5.0 to 7.0, the interaction between PASP and Cd²⁺, Zn²⁺ was found to become more effective especially at small f , because dissociation of carboxylate group becomes complete. Thus, at pH value higher than 5.0, relationship between i_d for Cd²⁺, Zn²⁺-PASP solutions and pH corresponds to that between the magnitude of $|\theta|_{222\text{nm}}$ for the same solution and pH at any f value.

As mentioned above, below pH 5.0 the dashed curves in Figs. 4-a and 4-b are superimposed on those with Cd²⁺, Zn²⁺ at various f values regardless of a decrease in i_d for both Cd²⁺ and Zn²⁺. The coordination mode of PASP and Cd²⁺, Zn²⁺ is, therefore, considered to be different from that at neutral pH one. In this lower pH region below 5, however, a certain number of carboxylate groups is already dissociated and sporadically distributed on PASP main chain since its pK_a is 4.2 ($I=1.0$) [19]. Although the dissociation of a pair of adjacent carboxylate group at the same time is favourable to the formation of helix structure by bivalent metal ion to cancel the negative charge of the group, the number of this mode of dissociation is, if any, statistically very small when dissociation is far from complete. Therefore, in this pH region below 3.5, Cd²⁺ or Zn²⁺ ion randomly bridges to any of those

sporadically dissociated groups in the helical part of PASP chain rather than to form further amount of helix structure and causes an aggregation of the PASP by the inter/intra bridging of PASP chain through Cd^{2+} , Zn^{2+} .

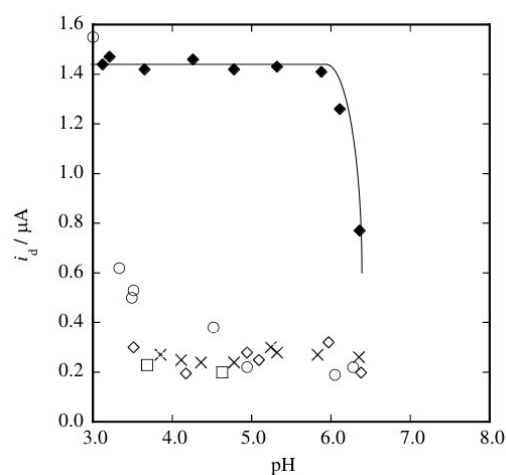


Fig. 5. The relationship between the decomposed diffusion current (i_d) for Pb^{2+} in PASP- Pb^{2+} solutions and pH at various values of f \blacklozenge ; $f=0$, \circ ; $f=2.09$, \square ; $f=3.13$, \diamond ; $f=4.17$, \times ; $f=6.26$

When this kind of coordination by an ion to PASP acts more effectively than by Cd^{2+} or Zn^{2+} , a much larger decrease in i_d will be observed.

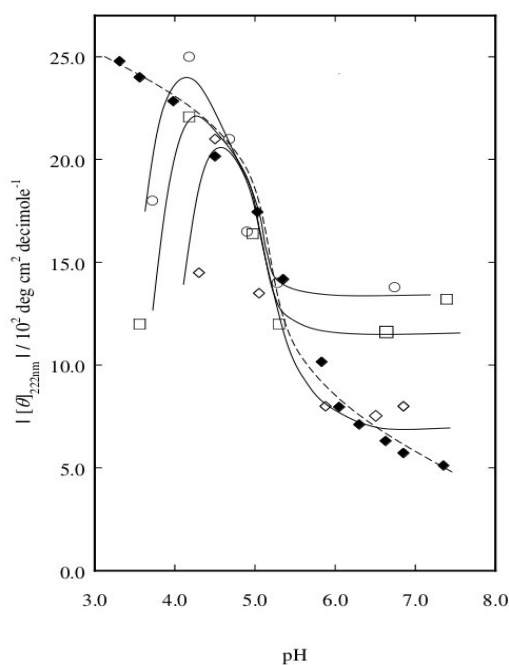


Fig. 6. The relationship between the molecular ellipticity at 222 nm for PASP- Pb^{2+} solutions and pH at various values of f . \blacklozenge , $f=0$, \circ ; $f=3.13$, \square ; $f=4.17$, \diamond , $f=5.21$

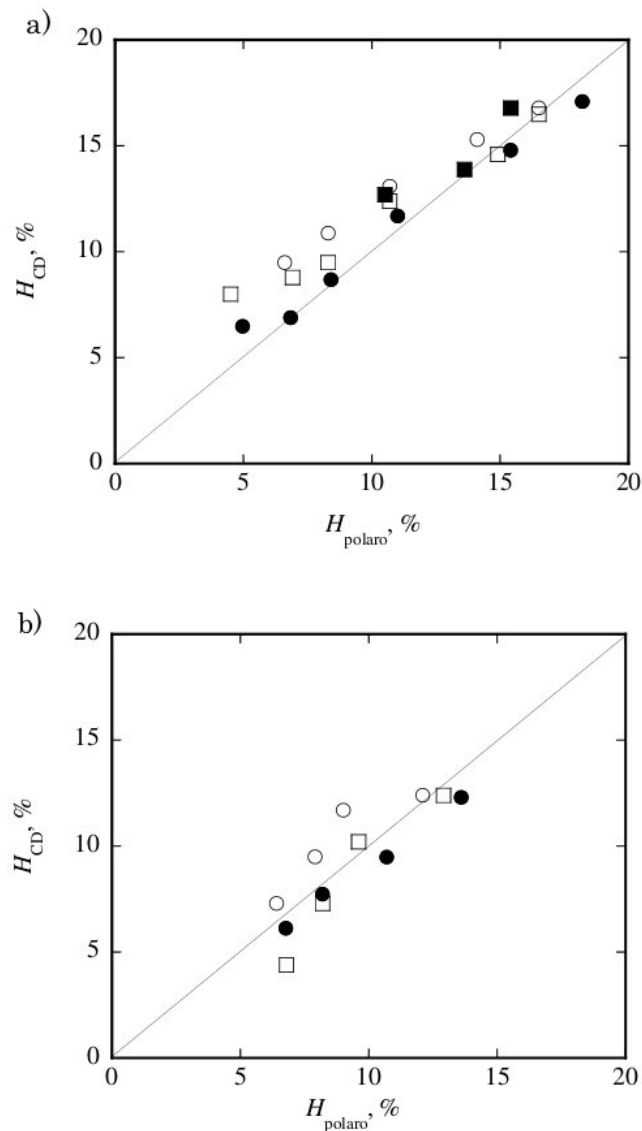


Fig. 7. (a) The relationship between the helix determined by polarography (H_{polaro}) and CD (H_{CD}) for PASP- Cd^{2+} solutions. ●; pH=7.0, □; pH=6.5, ○; pH=6.0, ■; pH=5.5. (b) The relationship between the helix content determined by polarography (H_{polaro}) and CD (H_{CD}) for PASP- Zn^{2+} solutions. ●; pH=7.0, □; pH=6.5, ○; pH=6.0

3.4. Diffusion current of Pb^{2+} and Molecular ellipticity at 222 nm

To elaborate on the coordination of mode in this pH region in more detail, we employed Pb^{2+} which is known to attach more strongly to carboxylate groups in PGA or PASP than other bivalent metal ion such as Cd^{2+} , Zn^{2+} , Co^{2+} [20]. Fig. 5 clearly shows the decrease in i_d for Pb^{2+} begins at much lower pH region and is more prominent compared to those for Cd^{2+} and Zn^{2+} in the corresponding pH region. The decrease in i_d of Pb^{2+} with $f=6.26$ is about twice as large as that for Cd^{2+} with $f=6.32$ at pH 3.5 and all the decreased i_d 's are almost independent of f . All the sample solution of containing Pb^{2+} at acidic pH region lower than

3.5 became turbid, characteristics for the aggregation of polymer [10]. CD spectra of the above solutions were also obtained. Although in the neutral pH region the same type of macromolecular complex by Pb^{2+} was found to exist like in the case of PASP-Cd^{2+} , Zn^{2+} , the magnitude of $[\theta]_{222\text{nm}}$ of PASP with Pb^{2+} begins to smaller than that of PASP without Pb^{2+} as pH becomes lower due to an excess amount of aggregation of polymers (or an occurrence of its precipitation) as shown in Fig. 6. These results for PASP-Pb^{2+} suggest that metal ion coordinates to PASP to cause an aggregation of polymers rather than to form helix structure in the lower pH region. The conformational change of PASP , therefore, sharply reflects on the voltammetric behaviour of PASP-metal ion system from lower acidic to neutral pH region.

3.5. Determination of helix content

Since the diffusion current of ion attached to the polymer is a parameter sensitive to the helix-coil transition, we have estimated the helix content in PASP by monitoring i_d on the assumption that all attachment of metal ion to PASP leads to the helix formation at around neutral pH region. Figs. 7-a and 7-b compare the helix content of PASP-Cd^{2+} , Zn^{2+} solutions determined by CD, H_{CD} , [11] and with that by i_d , H_{polaro} , at representative pH values. These techniques all give similar helix content values. This calculation was carried out as follows [21];

$$H_{\text{CD}} = \frac{|\theta]_{222\text{nm}} - 550}{(2400 - 550)} \times 0.27 \times 100 \quad (1)$$

$$H_{\text{polaro}} = \frac{(i_f - i_{\text{red}})}{i_f} \times 1/f \times 100 \quad (2)$$

Where i_f denotes the diffusion current without PASP and i_{red} is reduced current in the presence of PASP . $1/f$ is a normalizing factor for each solution eliminating the dependence on the concentration of PASP . The helix content due to Cd^{2+} or Zn^{2+} could be calculated separately even for these solution. The total helix content is easily obtained by either CD or a total decrease in i_d . The contribution of each ion to the total helix content could be estimated by comparing to the ratio of the decrease in i_d for Cd^{2+} or Zn^{2+} to that for solution without PASP . For example, at $\text{pH}=7.0$ and $f=6.26$, the helix content in PASP is 14.5% in the presence of both ions and the ratio of the helix content due to Cd^{2+} and Zn^{2+} was 0.55 and 0.45, respectively, when their relative concentration is 1:1. This result indicates that both Cd^{2+} and Zn^{2+} work almost equally in inducing the helix structure of PASP . Although only in a limited f and pH region, i_d is a useful parameter for the determination of helix content, the method proposed here could provide information on the helix content not only for PASP-single metal ion system but for systems which contain both Cd^{2+} and Zn^{2+} . It is also possible for other pairs of ions such as $\text{Cu}^{2+}/\text{Cd}^{2+}$, $\text{Cd}^{2+}/\text{Ni}^{2+}$, $\text{Zn}^{2+}/\text{Co}^{2+}$ or three kinds of ions among

these whose reduction potentials are large enough to be separated and also at any ratio of f . The results for these systems will be reported in due course.

3.6. Estimation of the size of macromolecular complexes

Diffusion co-efficient of molecules is an important parameter to provide information on a size or a shape of macromolecules and interaction between molecules and solvent. In Ilkovic's equation, diffusion coefficient for the complex ion, D_{complex} is expressed as follows;

$$D_{\text{complex}}^{1/2} = i_{d,\text{red}} / 607 n C m^{2/3} t^{1/6} \quad (3)$$

Where n is the number of electron transferred, C ; the concentration of the metal ion (mmol dm^{-3}), $m^{2/3} t^{1/6}$ ($=0.9445$), the capillary constant and $i_{d,\text{red}}$ is the diffusion current for PASP- Cd^{2+} , Zn^{2+} macromolecule complexes. Therefore, the size of the macromolecular-metal ion complex of PASP- Cd^{2+} and/or Zn^{2+} can be estimated with Stokes-Einstein's equation under an assumption that the complex can be regarded as a rigid sphere molecule [22,23].

$$r = (1/6\pi\eta)(RT/N)(1/D_{\text{complex}}) \quad (4)$$

Where r is the radius of the complex, R ; the gas constant, N ; Avoadro's number, T ; the absolute temperature (298 K), the viscosity of water (0.8904cP at 25°C) [24]. The size of the complexes of PASP- Cd^{2+} and Zn^{2+} can be obtained using eqns. 3 and 4. Table 1 shows the diffusion coefficients and radii of complexes and other pertinent data. The radius of complexes for helix content below 20% lies between *ca.* 16 and 36 Å. Muroga *et al.* estimated mean-square radius of gyration (r_g) for PASP (DP=100) by SAXS method [25]. The values for PASP with low or non-dissociated carboxlyate groups lie between 18 and 40 Å in aqueous or non-aqueous solution. Inoue *et al.* also reported the diffusion coefficient for partially helical PGA (DP=530) determined by polarography [23]. The value of that was $7.36 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$, which gives a radii of PGA 33 Å by eq. 4. Taking into account of difference in experimental conditions, results in our experiments are favourable to their values.

3.7. Estimation of the coordination number of Cd^{2+} , Zn^{2+} -PASP complex in the neutral pH region

If the reaction on the mercury electrode is reversibly by the reduction of a complex Cd^{2+} or Zn^{2+} to a metallic state at a dropping mercury electrode, then the following expression can be applied to obtain the coordination number, j , of such a complex as a given temperature. [26,27].

$$d(E_{1/2})/d(\log C_p) = -0.0591 \times j/n \quad (5)$$

Where C_p is the concentration of the free ligand (PASP) which can be approximated by the total concentration of PASP. The value of $E_{1/2}-E_{3/4}$ for Cd^{2+} is 0.027 V while that for Zn^{2+} is 0.020 V instead of 0.028 V as required for a two reversible reaction. The plot of $E_{1/2}$ for Cd^{2+} in the Cd^{2+} plus Zn^{2+} solution against $\log C_p$ at pH 7.0 is shown in Fig. 8. At this concentration of PASP, j was 1.93, 1.94, respectively, implying that approximately two peptide residue attach to one Cd^{2+} . This means electro-statically Cd^{2+} is supposed to coordinate to topologically two adjacent carboxylate groups in this pH region. Masujima and Yamaoka also pointed out the existence of this type of complex in the PGA- Cd^{2+} system using UV spectroscopy and the dialysis method [28].

Table 1. Helix contents, diffusion coefficient and radii for PASP- Cd^{2+} , PASP- Zn^{2+} , PASP- Cd^{2+} complexes at neutral pH region

Sample	pH	f [a]	H_{CD} , %	H_{polaro} , %	contribution of each ion	$i_{\text{d,complex}}$ / μs	i_{d} from each ion	$D_{\text{complex}} \times 10^{-7}$	r / \AA
PASP- Cd^{2+}	7	3.13	17.1	18.2		0.62		13.47	18.1
PASP- Cd^{2+}	7	4.17	14.8	15.4		0.52		9.48	25.9
PASP- Cd^{2+}	7	6.26	11.7	11.0		0.45		7.09	34.6
PASP- Cd^{2+}	7	8.34	8.7	8.4		0.44		6.79	36.2
PASP- Cd^{2+}	7	10.4	6.9	6.8		0.42		6.18	39.7
PASP- Zn^{2+}	7	4.17	12.3	13.6		0.64		14.36	17.1
PASP- Zn^{2+}	7	6.26	9.5	10.7		0.49		8.41	29.2
PASP- Zn^{2+}	7	8.34	7.7	8.2		0.47		7.74	31.7
PASP- Zn^{2+}	7	10.4	6.2	6.7		0.44		6.79	36.2
PASP- Cd^{2+} + Zn^{2+}	7	4.17	* 14.2	14.5	Cd 44.8 Zn 55.2	1.16	Cd 0.52 Zn 0.64	11.79	20.1
PASP- Cd^{2+} + Zn^{2+}	7	6.26	* 9.5	10.8	Cd 44.9 Zn 55.1	0.94	Cd 0.45 Zn 0.49	7.74	31.7
PASP- Cd^{2+} + Zn^{2+}	7	8.34	* 7.3	8.3	Cd 48.4 Zn 51.6	0.91	Cd 0.44 Zn 0.47	7.25	33.8
PASP- Cd^{2+} + Zn^{2+}	7	10.4	* 6.6	6.8	Cd 48.8 Zn 51.2	0.86	Cd 0.42 Zn 0.44	6.48	37.9
Cd^{2+}	3					1.45		69.9 (70.0 [b])	3.3(2.3 [c])
Zn^{2+}	3					1.48		73.7(72.0 [b])	3.3(2.1 [c])

[a] $f^* = 0.5[\text{PASP}]/[\text{Cd}^{2+}]$ or $[\text{Zn}^{2+}]$. [b] Ref. [28]. [c] Ref. [29]

4. CONCLUSIONS

Voltammetric technique is useful to study the helix-coil transition of PASP to which some reducible metallic ion are attached extrinsically as a marker. Although CD is a primarily tool to study the secondary structure of polypeptide, conformational transition of PASP does not reflect on CD spectral pattern so conspicuously as that of PGA. On the contrary, θ of the marker ions attached to PASP is sensitive to the conformational transition of PASP due to the formation of different types of macromolecular complexes depending on pH. In lower pH region PASP forms an aggregation of polymer chain by bridging of metal ion. In neutral one, PASP forms a helix structure by coordination with metal ion rather than that by hydrogen ion. Monitoring of the two ions for θ at a different reduction potential enables us to estimate each ion's contribution to the total helix content, respectively, even when more than single kind of ion exist. The diffusion co-efficient of macromolecular size of the complex is also evaluated by a decrease in θ . The technique in this report presents quantitative perspectives to the complex formation associated with the helix-coil transition using a metal ion as a marker in conjunction with CD measurements.

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