ISSN : 0974-7419 Volume 10 Issue 4

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 10(4) 2011 [239-245]

Voltammetric and CD spectroscopic studies of poly α-aspartic acid-cadmium ion complex in the helix-coil transition pH region

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Received: 26th August, 2010 ; Accepted: 5th September, 2010

ABSTRACT

Helix-coil transition of poly α-aspartic acid (PASP) was studied by dc polarography in the presence of Cd^{2+} as a marker attached to the polymer. The diffusion current of Cd^{2+} declined sharply in the pH range of 3.5-6.7 due to a formation of metal ion-PASP macromolecular complex. The complex formation also reflects on an increase of the magnitude at *ca*. 222 nm of CD spectrum, suggesting that PASP forms the helix structure by coordination of Cd^{2+} in the corresponding pH region. Two peptide residues coordinate approximately to one Cd^{2+} to form the helix structure in the neutral pH region by a shift of half-wave potential. Helix content determined by the decrease in diffusion current corresponds favorably to that by CD measurements. In the lower acidic pH region, the coordination mode of Cd^{2+} to PASP is different from that at neutral pH region. The decrease in diffusion current of Cd^{2+} is independent to the further formation of helix structure. Cd^{2+} ion coordinates with sparsely dissociated carboxylate groups of helical PASP which bring about an aggregation of polypeptide strands. The diffusion current of the ion attached to the polymer, therefore, is a parameter sensitive to a conformational change of PASP from acidic through neutral pH region.

KEYWORDS

Polarography;
Diffusion current;
Circular dichroism;
Helix-coil transition;
Poly aspartic acid-Cd^{2+} complex.

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 tertially 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 360-180 m region[9,10]. PGA is known to show the typical CD spectral pattern for helix structure or random coil state associated with this transition[8,9]. 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 spectral pattern by the transition of PASP is not so conspicuous as that 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[11,12,15]. In this paper, we employed voltammetric method to obtain electro-chemical perspectives to the helix-coil transition of PASP-metal ion complex using a cadmium ion as a marker.

EXPERIMENTAL

Materials

Sodium poly α-L-apsartic acid (PASP) (*M*w = 35,600) was purchased from Sigma Co. Stock solutions of Cd^{2+} were prepared from $3CdSO_4\ 8H_2O$ (Wako Pure Chemical Co. Ltd.). 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+} ion to PASP was defined as f (= [PASP] / [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. Reagents grade of sodium perchlorate was used as a supporting electrolyte at a concentration of 0.05 mol dm^{-3} for voltammetric measurements.

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

Apparatus

Voltammtric 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; $m = 0.863$ mgs^{-1}; $t = 1.26$ 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).

Effect of Hg column height on the limiting current

The effect of the mercury column height on the limiting currents for both helical and random coil PASP solutions containing Cd^{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} $NaClO_4$.

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 -1.4 V *vs* SCE indicating that the presence of PASP does not affect the electro-reduction process of Cd^{2+} or other metal ions.

RESULTS AND DISCUSSION

Polarograms and CD spectra of the solutions for PASP-Cd^{2+}

Figure 1 shows polarograms of Cd^{2+} in the presence and absence of PASP. The reduction of Cd^{2+} gives a well defined wave with a half wave potential of -0.50 V *vs* SCE. In the presence of PASP (f = 6.28), as pH increases from 3.67 to 6.80, the diffusion current (i_d) for Cd^{2+} decreases steadily and reaches to an almost constant value. On the other hand, i_d of Cd^{2+} without

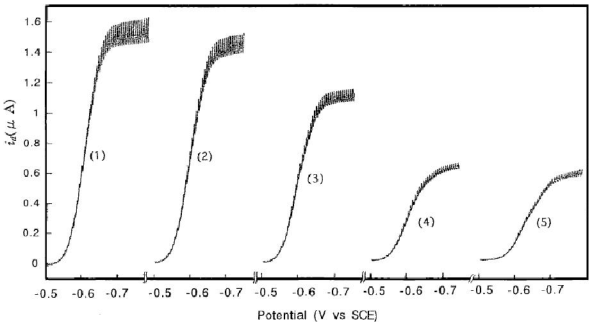

Figure 1 : pH dependence of polarograms for PASP-Cd^{2+} solutions. [Cd^{2+}] = 5.0×10^{-4} moldm^{-3}, f = 6.26. (1) pH = 3.67; without Cd^{2+}, (2) pH = 3.67, (3) pH = 4.30, (4) pH = 5.30, (5) pH = 6.80

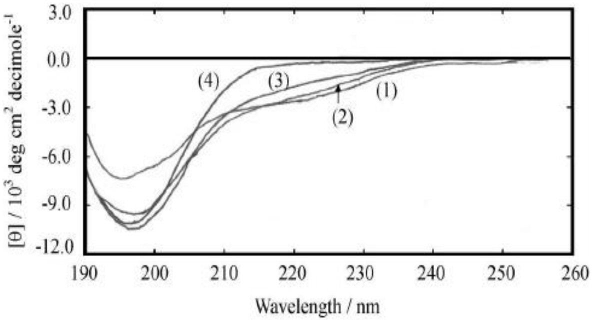

Figure 2a : pH dependence of CD spectra for PASP solutions. (1) pH = 3.60, (2) pH = 4.50, (3) pH = 5.35, (4) pH = 7.00

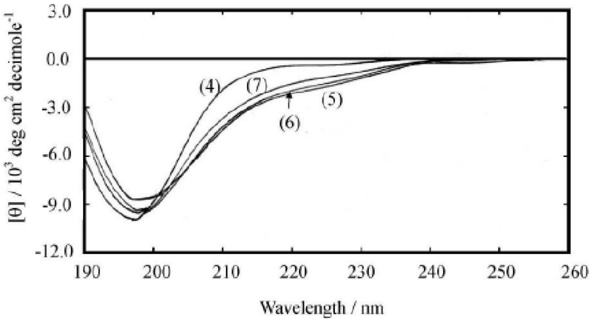

Figure 2b : Molecular ratio (*f*) dependence of CD spectra for PASP-Cd^{2+} solutions at pH = 7.0. (4) *f* = 0, (5) *f* = 4.17, (6) *f* = 6.26, (7) *f* = 8.34a

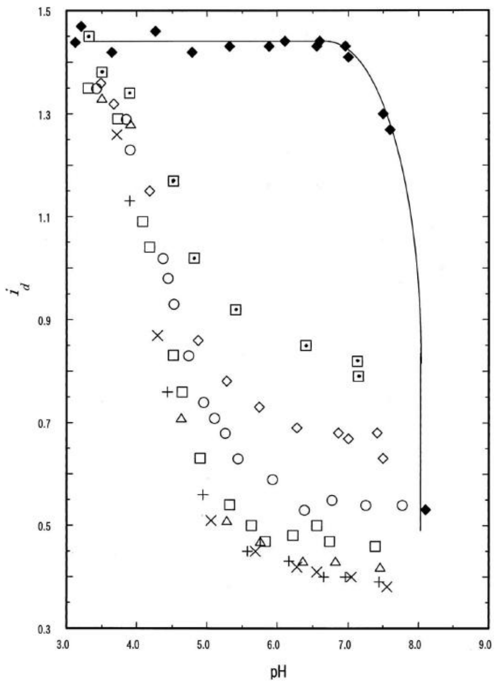

Figure 3 : Relationship between the diffusion current for Cd^{2+} in PASP-Cd^{2+} solutions and pH at various of *f*. ◆; without Cd^{2+}, ⊡; *f* = 2.09, ◇; *f* = 3.13, ○; *f* =4.17, □; *f* =6.26, △; *f* =8.34, +; *f* =10.4, ×; *f* =12.5

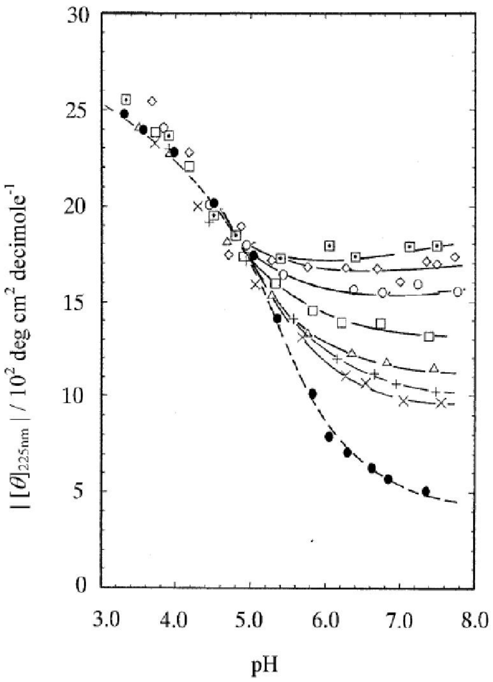

Figure 4 : Relationship between the molecular ellipticity at 225nm for PASP-Cd^{2+} solutions and pH at various values of *f*. •; without Cd^{2+}, ⊡; *f* = 2.09, ◇; *f* = 3.13, ○; *f* =4.17, □; *f* =6.26, △; *f* =8.34, +; *f* =10.4, ×; *f* =12.5

PASP remained unchanged in the same pH region. Figures 2-a and b show CD spectra of the above solutions. In solutions of neutral or alkaline pH region, PASP is known to be in a random coil state and has a CD spectrum with a very weak broad negative band at 230 nm and a very strong negative band at 198 nm[4,8,9]. In an acidic solution, although PASP tends to form the helix structure, its CD spectrum is 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[4,8,9]. As figure 2-a indicates, the magnitude of molecular ellipticity at 222 nm ($[\theta]_{222nm}$), corresponding to the helix content decreases with pH value. On the other hand, the CD spectra of all PSAP solutions in the presence of Cd^{2+} (f = 4.17 - 8.34) at pH 7.0 clearly show the same helix pattern as observed in figure 2-a and are essentially the same as those of PSAP obtained without any metal ion in the acidic pH region. These results indicate that Cd^{2+} plays an important role in the formation of α-helix structure of PASP in the neutral pH region.

Polarographic diffusion currents for solutions of PASP-Cd^{2+} at various mixing ratios

Figure 3 shows the relationship between i_d of Cd^{2+} and pH at various values of *f*. In the absence of PASP the diffusion current remains constant in the pH region from *ca*. 3.4 to 7.0. Above pH 7.4, i_d of Cd^{2+} decreased sharply due to a precipitation of $Cd(OH)_2$[16]. On the other hand, in the presence of PASP i_d of Cd^{2+} at various value of *f* decreased gradually from pH ca. 3.5. The decrease in diffusion current becomes large with increasing *f*, especially in the neutral pH region. This polarographic behavior means Cd^{2+} bounds to carboxylate groups within PASP to form α-helix structure, rendering the diffusion co-efficient of Cd^{2+} much smaller. The formation of helix structure is, therefore, indirectly revealed on the change in the value of diffusion current of Cd^{2+} which coordinates to PASP.

Molecular ellipticity at 222 nm for solutions of PASP-Cd^{2+}

The effect of pH on the magnitude of $[\theta]_{222nm}$ for the PASP-Cd^{2+} solutions with various values of *f* is shown in figure 4. The magnitude of molecular ellipticity [θ] at 222 nm is regarded as an indicator of α-helix content in PASP[11]. In the acidic pH region, the ionization of carboxylate group is almost suppressed to maintain the helix structure by hydrogen bonding between NH and CO groups in the peptide chain. As pH increases, carboxylate groups begin to dissociate protons, rendering PASP to the random state. This transition from helix structure to random coil state is quantitatively explained by tracing the dashed curve from acidic to neutral pH region in figure 4. The helix content of PASP (dashed curve) decreases linearly with an increase of pH value from 3.0 through 5.0, whether Cd^{2+} ion is present or not. In this pH region, therefore, Cd^{2+} ion does not contribute to the helix formation. From CD spectroscopic point of view, Cd^{2+} ion just only presents or attaches to carboxylate group without any contribution to the helix formation, although i_d of Cd^{2+} showed a decrease tendency in this pH region. Beyond pH 5.0, the magnitude of $[\theta]_{222nm}$ for PASP solution without Cd^{2+} decreases further to a value which shows 0 % helix structure at pH 7.8. When Cd^{2+} ion presents, however, beyond pH 5.0 the role of protons of the carboxyl sites on the helix structure is gradually replaced by Cd^{2+} so as to suppress the ionized groups. Thus, the deviation of those curves for the Cd^{2+}-PAPS solutions from dashed curve (PASP only) in the pH region of 5.0 - 7.9 begins to be observed. The degree of deviations of those curves from the dashed one becomes large with an increase in the ratio of Cd^{2+} ion to [PASP]. In the pH region (*ca*. 5.0 - below 7.0), the helix formation is sustained not only by H^+ but also by Cd^{2+} which is randomly distributed on the carboxylate groups. Above pH 7.0 PASP forms the helix structure not by H^+ but by Cd^{2+} which directly correlate with the decrease in i_d. As pH increases from 5.0 to 7.0, the interaction between PASP and Cd^{2+} was found to become more effective especially at small *f*, because the dissociation of carboxylate group become complete. Thus, at pH values higher than 5.0, the relationship between i_d of PASP- Cd^{2+} solutions and pH corresponds to that between the magnitude of $[\theta]_{222nm}$ for the same solutions and pH at any *f* value. As mentioned above, below pH 5.0, Cd^{2+} does not contribute to the further helix formation of PASP, even though i_d of PASP-Cd^{2+} solutions are always smaller than that without PASP in the corresponding pH region. The coordination mode of PASP and Cd^{2+} is, therefore, considered to be different from that at neutral pH region. Below pH = 5.0, a certain number of carboxylate groups is already dissociated since its p*K*a is 4.2 (I = 1.0)[20]. The number of dissociated carboxylate groups, however, is smaller than that at neutral pH region on PASP so that the ionized sites are sporadically distributed on PASP chain. Although the dissociation of a pair of adjacent carboxylate group at the same time is favorable to the formation of helix structure by bivalent metal ion, the number of this mode of dissociation is, if any, statistically very small. Therefore, especially in the lower pH region (pH < 3.5), Cd^{2+} ion cannot help but bridge to these sporadically dissociated groups in the helical part of PASP chains rather than further formation of helix structure, causing aggregation of the PASP by the inter/intra bridging of PASP chain through Cd^{2+}. On the while, a few Cd^{2+} coordinates to contribute the helix formation (pH 3.5-5.0). The conformational change of PASP, therefore, sharply reflects on the voltammetric behavior of metal ion-PASP solution from the lower acidic through neutral pH region.

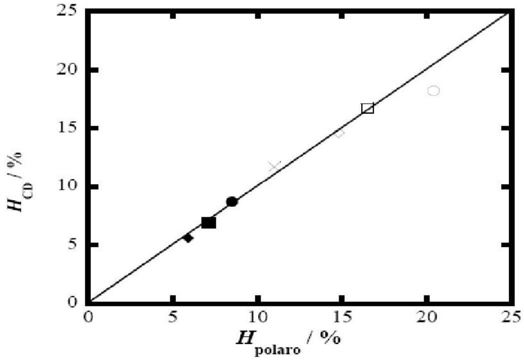

Figure 5 : Relationship between the helix determined by polarography (H_{polaro}) and CD (H_{CD}) at pH = 7.0 for PASP-Cd^{2+} solutions. ○; *f* =2.09, □; *f* =3.13, ◇; *f* =4.17, ×; *f* =6.26; *f* =8.34, ■; *f* =10.4, ◆; *f* =12.5

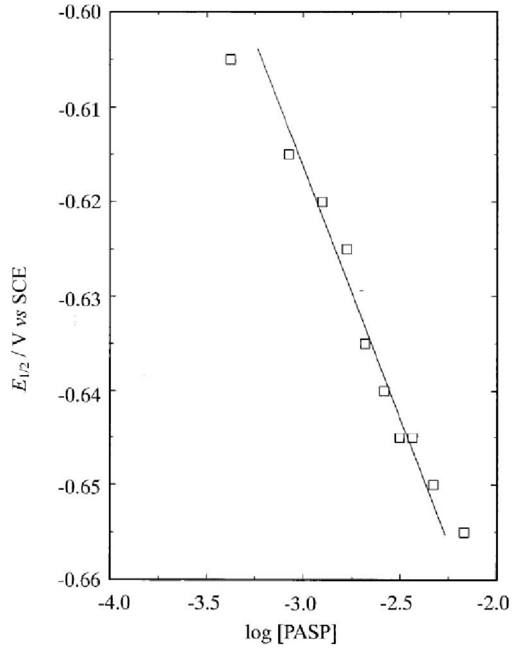

Figure 6 : Relationship between the half-wave potential for Cd^{2+} and the concentration of PASP at pH 7.0

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 the diffusion current on the assumption that all attachment of metal ion to PASP leads to the helix formation at around neutral pH region. Figure 5 compares the helix content of PASP-Cd^{2+} solutions determined by CD, H_{CD}, and with that by diffusion current, H_{polaro} at representative pH values. These techniques all give similar helix content values. This calculation was carried out as follows[18];

TABLE 1 : Helix contents, diffusion coefficients and redii for PASP-Cd^{2+} complexes at pH 7.0

Sample	*f*	$Helix_{CD}$/%	$Helix_{polar}$/%	$i_{d,complex}$/μA	$D_{complex}\times10^7$/ cm^2s^{-1}	$r_{complex}$/Å
PASP-Cd^{2+}	2.09	18.2	20.4	0.85	25.3	10.6
PASP-Cd^{2+}	3.13	16.7	16.5	0.70	17.2	14.2
PASP-Cd^{2+}	4.17	14.6	14.8	0.55	10.6	23.0
PASP-Cd^{2+}	6.26	11.7	11.0	0.45	8.07	30.2
PASP-Cd^{2+}	8.34	8.7	8.5	0.42	7.09	34.4
PASP-Cd^{2+}	10.4	6.9	7.1	0.38	6.78	35.9
PASP-Cd^{2+}	12.5	5.6	5.9	0.38	6.48	37.6
Cd^{2+}	-	-	-	1.44	69.9(70.0[a])	3.3(2.3[b])

[a]Ref. 24, [b]Ref. 25

$$\mathbf{H_{CD} = \frac{\left|[\theta]_{225nm}\right| - 550}{2400 - 550} \times 0.27 \times 100} \quad (1)$$

$$\mathbf{H_{polaro} = \frac{i_{d,free} - i_{d,red}}{i_{d,free}} \times \frac{1}{f} \times 100} \quad (2)$$

where i_f denotes the diffusion current without PASP and i_{red}, the reduced current in the presence of PASP. 1/*f* is a normalizing factor for each solution eliminating the dependence on the concentration of PASP.

Both results are comparable to each other in neutral pH region. Although circular dichroism has been the most common method in determination of helix content of poly amino acid[8,9], voltammetry also is found to be an auxiliary method for the determination of helix content by using metal ion as a marker.

Estimation of the coordination number of PASP-Cd^{2+} complexes in the random coil pH region

If an electrode reaction proceeds reversibly by the reduction of a complex Cd^{2+} ion to a metallic state at the dropping mercury electrode, then the following expression can be applied to obtain the coordination number, *j*, of such a complex at a given temperature[19].

$$\mathbf{\frac{d(E_{1/2})}{d(\log C_x)} = -j\frac{0.059}{n}} \quad (3)$$

Thus, from the slope of the $E_{1/2}$-log C_x curve, a value of *j* can be evaluated. Where C_x is the concentration of the free ligand (PASP) which can be approximately by the total concentration of PASP. The value of $E_{3/4}$-$E_{1/2}$ for Cd^{2+} is 0.027 V, required for a two electron reversible reaction. The plot of $E_{1/2}$ for Cd^{2+} against $\log C_x$ at pH 7.0 is shown in figure 6. At this concentration of PASP, j was 1.86, implying that approximately two peptides residues attach to one Cd^{2+} adjacent carboxylate groups in this pH region[20].

Estimation of the size of macromolecular complexes

The size of the macromolecules complexes of PASP-Cd^{2+} can be estimated with Stokes-Einstein equation under an assumption that the complex can be regarded as a rigid sphere molecules[21,22].

$$\mathbf{r} = \frac{1}{6\pi\eta}\frac{RT}{N}\frac{1}{D_{complex}} \qquad (4)$$

where r is the radius of the complex; R is the gas constant; N is Avogadro's number; $D_{complex}$, the diffusion coefficient for the complex ion; T, the absolute temperature(at 298K); η, the viscosity of water (0.8904 cP at 25 °C)[27] in the Ilcovič equation, $D_{complex}$ is expressed as follows;

$$D_{complex^{1/2}} = \frac{i_{d,complex}}{607\,n\,C\,m^{2/3}\,t^{1/6}} \qquad (5)$$

where n is the number of electrons transferred, C is the concentration of the metal ion (mmol dm^{-3}), $m^{2/3}\,t^{1/6}$ (0.9445) is the capillary constant, and $i_{d,complex}$ is the diffusion current for PASP-Cd^{2+} macromolecular complex at pH 7. We employed i_{red} in eq. (2) as $i_{complex}$, since a reduction of Cd^{2+} is due to the complex formation by PASP.

The size of the complexes of PASP-Cd^{2+} can be obtained by using eq. (4) and (5). TABLE 1 shows the diffusion coefficients and radii of the complexes and other pertinent data. As long as PASP has a relatively high fraction of helix, the radius of the complexes lies between 10 and 45 Å in a helix content below 20 %. These values are comparable to those obtained from SAXS method for PASP (M_w =10,500) proposed by Muroga et al.[23] Their values of root mean square radius of gyration (r_g) for PASP lies between 18 and 48 Å. Taking into account of the difference in instrumental method, the results in this experiment are favorable to their values. It is of interest to be able to evaluate easily the size of macromolecule-metal ion complex by electrochemically using Cd^{2+} ion as a marker.

CONCLUSIONS

We have shown that voltammetry is a useful tool in the study of the helix-coil transition of PASP to which some reducible metallic ions are attached extrinsically as a marker. Although CD is a primarily method for studying the secondary structure of poly peptide, conformational change of PASP does not reflect on CD spectrum so conspicuously as that of PGA. In the pH range 5-7.9, i_d of Cd^{2+} attached to PASP changes sensitively in proportion to a formation of helix structure by PASP, while in the lower pH region, the decrease is due to the aggregation of PASP helical strands. The polarograhic diffusion current is, therefore, a parameter sensitive to the conformational change of PASP in the low acidic to neutral pH region. The metal ion attached to PASP works as a probe to investigate this transition of PASP and provides quantitative information on this transition such as helix content of PASP, coordination number of metal ion to PASP residue, sizes of the complex, and binding mode of complex from low acidic to neutral pH region. Voltammetric technique, therefore, works as an auxiliary tool to provide a different perspective to the helix-coil transition of PASP in conjunction with CD measurements.

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