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Effects of Zinc on the Activity and Conformational Changes of Arginine Kinase and Its Intermediate

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The effects of zinc on arginine kinase and its collapsed-state intermediate were studied. Both arginine kinase and the collapsed-state intermediate were inactivated in the presence of zinc, following a biphasic kinetic course. The corresponding apparent rate constants of inactivation at different zinc concentrations and conformational changes in the presence of 0.5 mM zinc were obtained. The conformational changes of arginine kinase and the collapsed-state intermediate were followed by fluorescence spectra and circular dichroism spectra. Comparison of the results for arginine kinase and the collapsed-state intermediate showed that the collapsed-state intermediate was more susceptible to zinc, which indicated that the collapsed-state intermediate was more flexible and unstable than arginine kinase. The special structure of arginine kinase might explain these diverse phenomena.

Keywords: Arginine kinase, Collapsed-state intermediate, Conformational change, Inactivation, Zinc

Introduction

Arginine kinase (AK, E.C.2.7.3.3) usually exists as a monomeric enzyme (M_r 40 kDa) that catalyzes the bimolecular reaction (Zhou *et al*., 1998):

$$\text{arginine} + \text{MgATP}^{2-} \rightleftarrows \text{MgADP}^{-} + \text{phosphoarginine}^{2-} + \text{H}^{+}$$

The enzyme plays a key role in the interconnection of energy production and utilization in invertebrates, which is similar to the role of its homologous enzyme creatine kinase (CK, E.C.2.7.3.2) in vertebrates (Suzuki *et al*., 1997; Zhou *et al*., 1998). The crystal structure of horseshoe crab *limulus* AK (Zhou *et al*., 1998) was solved by the X-ray diffraction method. The inactivation and conformational changes of AK in various denaturants, including guanidinium chloride (Gross *et al*., 1995) and urea (France and Grossman, 1996), were investigated. In the unfolding study of AK that is induced by urea, a collapsed-state intermediate (CSI) with some features of molten globule was found in 0.5 M urea. It was conceptualized as possessing a compact, native-like secondary structure and a fluctuating, more denatured-like tertiary structure, but with catalytic activity retention, which was even higher than the native enzyme (France and Grossman, 1996). It was reported that similar conformational isoforms existed in the native solvent environment (Grossman, 1991). These transition-state structures appeared to be relevant as kinetic intermediates during the folding process of proteins, not only *in vitro* (Ptitsyn *et al*., 1990) but also *in vivo* (Martin *et al*., 1991; Sundd *et al*., 2002).

Zinc is an essential metal element for the regulation of a great variety of biological processes in animal cells (Berg and Shi, 1996). Because of its specific electron configuration, zinc possesses four distinct characteristics apart from other divalent cations, which may contribute to its importance in life (Berg and Shi, 1996). First, when coordinated by ligands in any geometry, Zn^{2+} has stereochemical flexibility (Berg and Shi, 1996). Second, in terms of the hard-soft acid-base theory, Zn^{2+} has amphoteric property (Berg and Shi, 1996). Third, divalent zinc has no redox activity (Vallee and Auld, 1995). Finally, Zn^{2+} has chemical stability, and it rapidly undergoes the ligand exchange reaction (Berg and Shi, 1996). However, although an authentic mechanism has not been well established, evidence has shown that zinc is toxic in living organisms (Schmalz *et al*., 1997; Tong *et al*., 2000). Zinc can induce hydrophobic exposure and/or aggregation of many proteins, including creatine kinase (CK, E.C.2.7.3.2), which may occur in Alzheimer's disease (Tong *et al*., 2000).

The investigation of intermediates is useful in the study of

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Abbreviations: AK, arginine kinase; CK, creatine kinase; CD, circular dichroism; CSI, collapsed-state intermediate; ANS, 8-anilino-1-naphthalene-sulfonic acid; SDS, sodium dodecyl sulfate.

protein folding. However, some intermediates can even exist *in vivo* (Martin *et al*., 1991; Sundd *et al*., 2002); therefore, zinc in the cell will inevitably affect the conformation and activity of AK and its intermediate. Nevertheless, there has been little research performed, that correlates the structural changes of AK to the changes in its catalytic activity under the effects of zinc. In particular, the effects of zinc on the intermediates of AK have not been previously reported. Studies on the effects of zinc on CK (Tong *et al*., 2000) found that at low zinc concentrations conformational changes were seriously affected with little change in activity. The inactivation and conformational changes of AK and one of its intermediates, which were induced by zinc, and obtained in this study differed from those of CK. This paper also discusses the potential biological significance of these effects.

Materials and Methods

Materials AK, arginine, 8-anilino-1-naphthalene-sulfonic acid (ANS), G-75, DEAE-cellulose, urea, and ATP were purchased from Sigma (St. Louis, USA). Acrylamide, ammonium persulfate, and N,N,N',N'-tetramethyl ethylenediamine were purchased from BioRad (Hercules, USA). N'N'-methylene-bis-acrylamide and sodium dodecyl sulfate (SDS) were BDH products. β-Mercaptoethanol and glycine were from Ameresco. All of the other reagents were local products of analytical grade without further purification.

Methods

Further purification of AK The enzyme from Sigma was dissolved in a minimum volume of a native buffer (100 mM glycine-NaOH containing 10 mM β-mercaptoethanol, PH 8.0) and applied to a DEAE-cellulose column (2.5×10 cm^2) that was equilibrated with the native buffer. The column was washed with the same buffer, and then eluted with a linear gradient of 0-500 mM NaCl. The fraction that possessed AK activity was pooled and applied to a Sephadex G-75 column (1.5×25 cm^2) that was equilibrated with a native buffer. The column was eluted with a native buffer. The fraction that possessed AK activity was pooled and frozen to powder. The purified enzyme was proven homogeneous by using both native polyacrylamide gel electrophoresis (PAGE) and SDS-PAGE.

Enzyme concentration and activity assay The concentration of AK was determined by absorbance at 280 nm with $A1\%_{1cm} = 6.7$ (France and Grossman, 1996). AK activity was measured using the colorimetric method that was described by France and Grossman (1996), except that all of the reagents used were dissolved in a native buffer, and the temperature for the reaction was 30ºC. The concentration and activity of AK were measured with an Analytikajena Specord 200 UV/VIS Spectrometer. The specific activity of AK using this colorimetric assay was 50.0 μmol phosphate/min/mg.

The preparation and activity assay of CSI When AK was incubated in a CSI buffer (native buffer containing 0.5 M urea) for 1 h, then CSI could be obtained (France and Grossman, 1996). The CSI activity was assayed by the previously described method, except that all the reagents used in the assay activity were dissolved in a CSI buffer.

Inactivation of AK or CSI by Zn^{2+} and residual activity assay For the assays in different zinc concentrations, AK and its CSI were incubated with different concentrations of zinc at 30ºC for 60 min. The assays for these samples were the same as those previously described, except that the reagents also contained the same concentration of zinc that was used to treat the protein.

Measurement of inactivation kinetic course The kinetic courses of AK (1 μM) and its CSI (1 μM) inactivation were monitored through measurements of AK and its CSI activities at different time intervals in a native buffer and CSI buffer, respectively, with $ZnCl_2$ at the desired concentration (30ºC).

Fluorescence spectroscopy The AK and CSI concentrations were 4.5 μM and the ANS concentration in the ANS fluorescence spectroscopy measurement was 50 μM. All of the measurements were performed and corrected (using the spectrum of each buffer solution) using a Hitachi 850 Spectrofluorometer. The excitation wavelengths of the intrinsic fluorescence and ANS fluorescence were 295 nm and 400 nm, respectively. The measurements were done in each buffer with $ZnCl_2$ at the desired concentration (30ºC). The intrinsic and ANS fluorescence spectroscopy of AK and CSI at different zinc concentrations were measured after adding zinc to AK and CSI for 60 min at 30ºC.

The kinetic courses of ANS fluorescence intensity changes of AK (4.5 μM) and its CSI (4.5 μM) were monitored through measurements of the fluorescence intensity changes at 462 nm (the excitation wavelength was 400 nm) at different time intervals in a native buffer and CSI buffer, respectively, with $ZnCl_2$ at the desired concentration (30ºC).

Circular dichroism (CD) spectroscopy Far ultra violet CD spectra of AK and CSI at different zinc concentrations were recorded from 250 nm to 200 nm, on a Jasco 715 spectropolarimeter with a 2 mm light path cell after adding zinc to AK and CSI for 60 min in each buffer at 30ºC. The AK and CSI concentrations were 4.5 μM each. All the spectra data were corrected with blanks.

The kinetic courses of 222 nm ellipticity changes of AK (4.5 μM) and its CSI (4.5 μM) were monitored by measuring the ellipticity changes at different time intervals in a native buffer and CSI buffer, respectively, with $ZnCl_2$ at the desired concentration (30ºC).

Measurements of AK/CSI aggregation The AK/CSI aggregation was measured by following the absorbance change at 400 nm on an Analytikajena Specord 200 UV/VIS Spectrometer in each buffer with $ZnCl_2$ at the desired concentration (30ºC). The cell diameter was 1 cm.

Results

Inactivation of native AK and its CSI by Zn^{2+} The

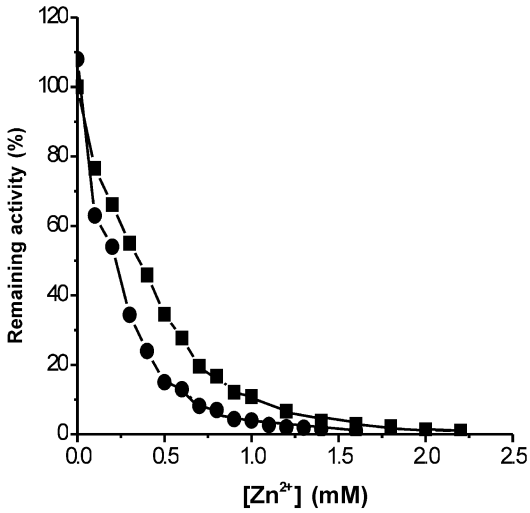

Fig. 1. Effect of Zn^{2+} on the residual activity of AK (■) and its CSI (●). The enzyme and intermediate were incubated in each buffer for 1 h at 30°C with different concentrations of $ZnCl_2$. The concentrations of AK and its CSI were 4.5 μM each.

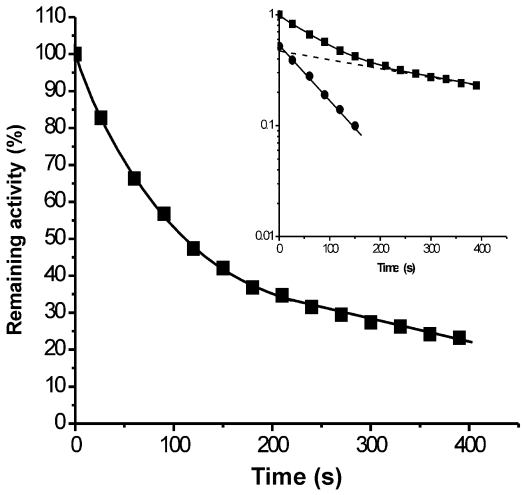

Fig. 2. Kinetic course of AK inactivation induced by zinc. The zinc concentration was 0.3 mM, the AK concentration was 1 μM, and the temperature was 30°C. The insert shows a semilogarithmic plot. Experimental data (■). Points obtained by subtracting the contribution of the slow phase (●).

residual activities of both AK and its CSI after incubation with zinc at 30°C for 1 h decreased as the zinc concentration increased (Fig. 1). However, the remaining CSI activity decreased more than the remaining AK activity in the presence of the same concentration of Zn^{2+}. The remaining activity of AK and its CSI at 0.1 mM Zn^{2+} were 77% and 63% of native AK activity, respectively; at 1 mM Zn^{2+} the corresponding values were 11% and 4%, respectively. AK was completely inactivated when the Zn^{2+} concentrations exceeded 1.5 mM; however, no obvious CSI activity was observed at Zn^{2+} concentrations beyond 0.9 mM. The inactivation midpoint occurred at about 0.35 mM Zn^{2+} in native AK and 0.2 mM in CSI. A control experiment showed that the amounts of zinc that were used in Fig. 1 had little effect on the activity measurements. Under our conditions, the CSI activity reached 108% of the native AK activity in the absence of zinc. A parallel experiment, using NaCl at different concentrations as controls, showed that even with NaCl concentration up to 100 mM, there was no significant effect on AK and its CSI activities (data not shown). Therefore, the changes in the residual activities of AK and its CSI in the presence of $ZnCl_2$ were not due to Cl^- and the resulting changes in the ionic strength, but due to Zn^{2+}.

Kinetic course of AK or CSI affected by Zn^{2+} The inactivation course of AK that was incubated with 0.3 mM zinc at 30°C (Fig. 2) showed that the inactivation followed a biphasic kinetic course. As shown in the inset of the semilogarithmic plot, the curve can be resolved into two straight lines to obtain the kinetic constants of the fast and slow phases, respectively. Using the same method, the kinetic constants for AK inactivation in the presence of 0.45 mM, 0.7 mM, and 1 mM zinc were also obtained. The results are summarized in Table 1. The inactivation kinetic courses for CSI at different zinc concentrations, 0.3 mM, 0.45 mM, 0.7 mM, and 1.0 mM, were measured with the same method in a CSI buffer. The rate constants are also summarized in Table 1. With the zinc concentration increase, both the fast phase rate constant (k_1) and slow phase rate constant (k_2) of native AK increased, but k_1 increased faster. For CSI, the fast phase rate constant (k_1) significantly increased with the increase of zinc concentration, while the slow phase rate constant (k_2) only changed a little. From Table 1, it can be seen that the rate constants, k_1 and k_2, of CSI are higher than those of native AK.

Table 1. Summary of rate constant of inactivation of AK (1 μM) and its CSI (1 μM) during zinc denaturation at 30°C

[Zinc] (mM)	Rate constant (s^{-1}) of AK		Rate constant (s^{-1}) of CSI	
	k_1 ($\times 10^3$)	k_2 ($\times 10^3$)	k_1 ($\times 10^3$)	k_2 ($\times 10^3$)
0.3	2.49	0.62	3.11	1.16
0.45	3.55	0.78	5.75	1.21
0.7	5.17	0.88	7.69	1.35
1.0	7.33	1.24	8.64	1.20

The kinetic courses of conformational change were monitored by ANS fluorescence spectrum and CD spectrum in the presence of 0.5 mM zinc (Figs. 3 and 4). The ANS fluorescence intensity change (Fig. 3) and the 222 nm ellipticity change (Fig. 4) followed a biphasic kinetic course with the curve that was resolved into two straight lines in the semilogarithmic plot. The rate constants for inactivation, ANS fluorescence intensity, and 222 nm ellipticity changes in the presence of 0.5 mM zinc are listed in Table 2. The inactivation

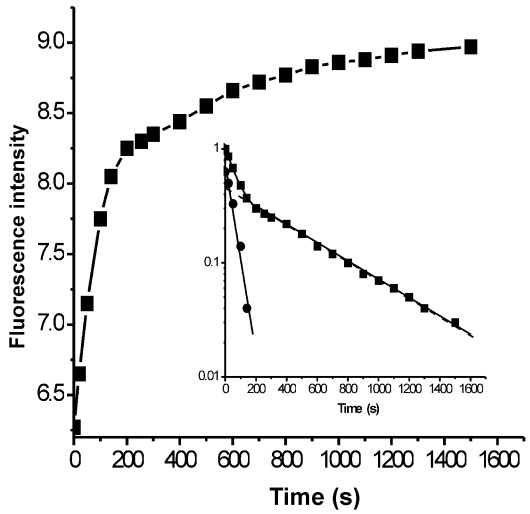

Fig. 3. Kinetic course of AK's (4.5 μM) ANS fluorescence intensity change in the presence of 0.5 mM zinc. The inset shows a semilogarithmic plot. Experimental data (■). Points obtained by subtracting the contribution of the slow phase (●).

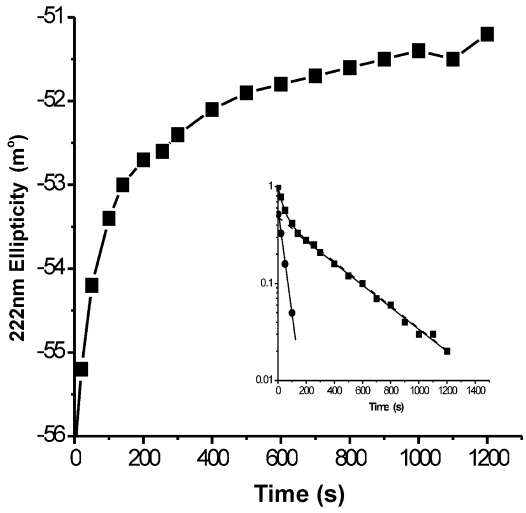

Fig. 4. Kinetic course of AK's (4.5 μM) 222 nm ellipticity change in the presence of 0.5 mM zinc. The inset shows a semilogarithmic plot. Experimental data (■). Points obtained by subtracting the contribution of the slow phase (●).

rate constants of AK and its CSI, k_1 and k_2, are higher than the conformational change rate constants. However, the inactivation and conformational change rate constants of AK are lower than those of CSI (Table 2).

Conformational change

CD Spectroscopy The CD spectra for AK and CSI at different zinc concentrations are shown in Fig. 5. As the zinc concentration increased, the AK ellipticity decreased. The decrease in ellipticity at 222 nm indicated that the α-helix content in AK also decreased. The CSI result was similar to that of AK, but the ellipticity changes of CSI were more significant. However, the 222-nm ellipticity showed that the α-helix content of CSI was higher than that in native AK in the absence of zinc.

ANS fluorescence The influences of Zn^{2+} on the exposures of AK and its CSI hydrophobic surfaces were probed by measuring the fluorescence that was associated with the ANS binding (Fig. 6). When the zinc concentration increased from 0 mM to 3.5 mM, then the ANS emission fluorescence intensity of native AK and its CSI increased from 6.28 to 13.29 and from 7.63 to 14.48, respectively. This was accompanied by a corresponding blue shift of the emission peak from 464 nm to 462 nm and from 465 nm to 461 nm. These results indicate that AK and its CSI have exposure to some extent on their hydrophobic surfaces, and that CSI had greater changes. However, compared with native AK, ANS fluorescence emission intensity for CSI reached 121% of the value for native AK in the absence of zinc.

Intrinsic fluorescence Intrinsic fluorescence was also used to follow AK and its CSI conformational changes at different zinc concentrations (Fig. 7). The results were similar to the results of the ANS fluorescence spectra. The intrinsic fluorescence maximum of native AK and its CSI red shifted from 331 nm to 335 nm and from 332 nm to 337 nm, respectively, accompanied by a corresponding fluorescence intensity that increased from 36.94 to 57.75 and from 48.8 to 68.52, with the zinc concentration increasing from 0 mM to 3.5 mM. These results indicate that the tryptophan residues moved, on average, to a more polar environment. Also, CSI had greater changes.

Table 2. AK and its CSI inactivation[a] and conformational change[b] rate constants

	Rate constant (s^{-1}) of AK		Rate constant (s^{-1}) of CSI	
	k_1 ($\times 10^3$)	k_2 ($\times 10^3$)	k_1 ($\times 10^3$)	k_2 ($\times 10^3$)
Inactivation	3.55	0.78	5.75	1.21
ANS fluorescence intensity change	2.23	0.28	3.17	0.44
222-nm ellipticity change	2.78	0.32	3.34	0.51

a: The zinc concentration was 0.5 mM and the enzyme concentration was 1 μM.
b: The zinc concentration was 0.5 mM and the enzyme concentration was 4.5 μM.

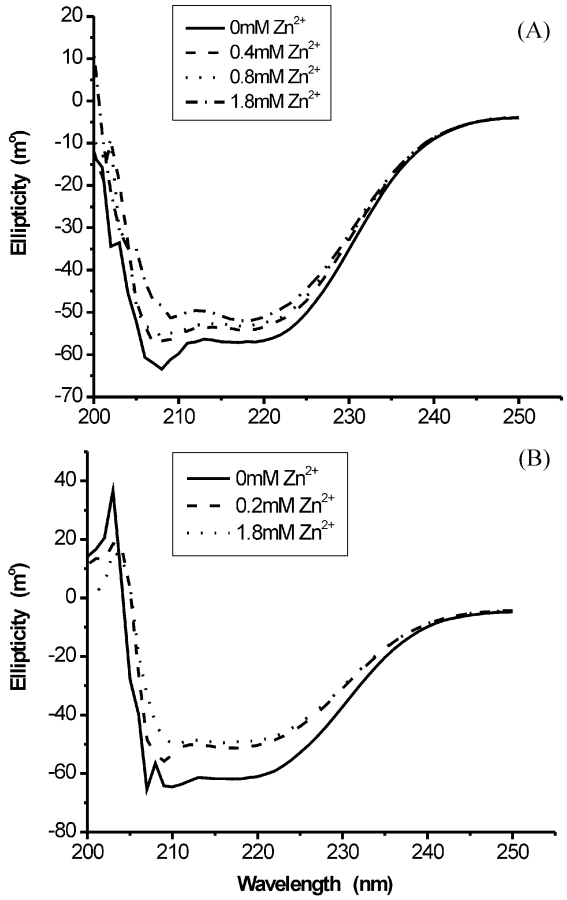

Fig. 5. (A) Far ultra violet CD spectra of AK (4.5 μM) in the presence of different zinc concentrations at 30°C. (B) Far ultra violet CD spectra of CSI (4.5 μM) in the presence of different zinc concentrations at 30°C.

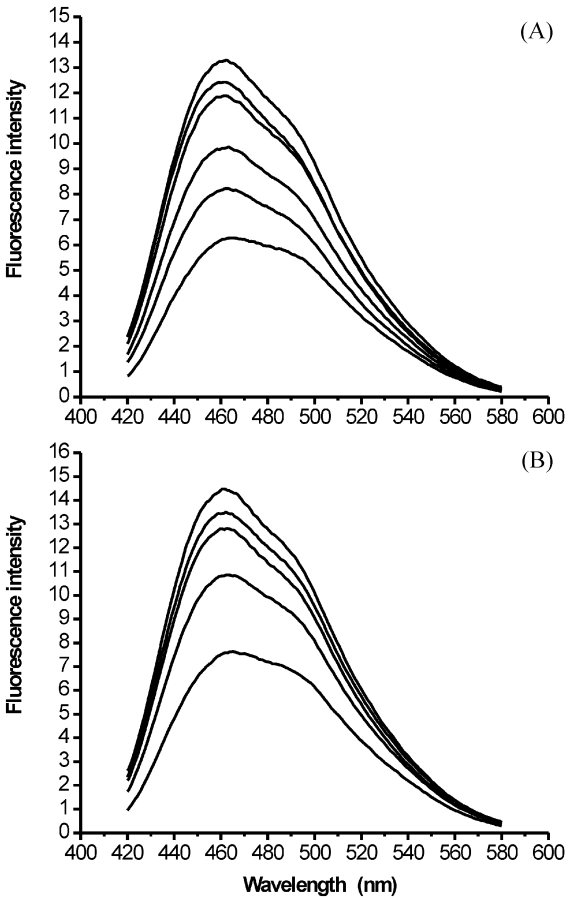

Fig. 6. (A) ANS fluorescence emission spectra of native AK (4.5 μM) in the presence of different zinc concentrations at 30°C. The zinc concentrations were 0 mM, 0.4 mM, 0.6 mM, 1.2 mM, 2.0 mM, and 3.5 mM from the bottom upward. (B) ANS fluorescence emission spectra of CSI (4.5 μM) in the presence of different zinc concentrations at 30°C. The zinc concentrations were 0 mM, 0.2 mM, 0.6 mM, 1.0 mM and 3.5 mM from the bottom upward.

Aggregation Under our experimental conditions, when AK or its CSI concentration was below 5 μM and the zinc concentration was below 5 mM, then there were no detectable aggregations (data not shown). Therefore, the inactivation of AK and its CSI in the presence of Zn^{2+} were not due to protein aggregation, but to other reasons (see the following discussion).

Discussion

The relationship between structure and function is one of the central issues in the investigation of biological macromolecules. The conformational integrity of an enzyme is essential for its activity (Tanford, 1968). Previous authors largely concentrated on the unfolding and refolding of enzymes under urea and guanidinium chloride with relatively few attempts to explore the effects of metal ions on the unfolding of protein. However, under various physiological conditions and solvents that contain different kinds and concentrations of metal ions, the enzyme conformation and thus its activity can be affected by the interaction with these ions (Tong *et al*., 2000). In this investigation, the results showed that even in a dilute solution, zinc could strongly inhibit AK activity, but with few conformational changes (Table 3). It is evident that the zinc concentration for AK's conformational change is higher than that for activity inhibition, similar to the result from the unfolding study of AK by urea or guanidinium chloride (Gross *et al*., 1995). The kinetic results showed that the inactivation rate constants were higher than the conformational change rate constants (Table 2). These evidences may indicate that the active sites of AK are probably located in a relatively fragile part of the enzyme. Hence a slight disturbance in the spatial arrangement of the active site can destroy the enzyme activity before any gross conformational change can be detected (Tsou, 1993; Tsou, 1998). A comparison with the result from CK reveals that the effects of zinc on AK at low concentrations are much

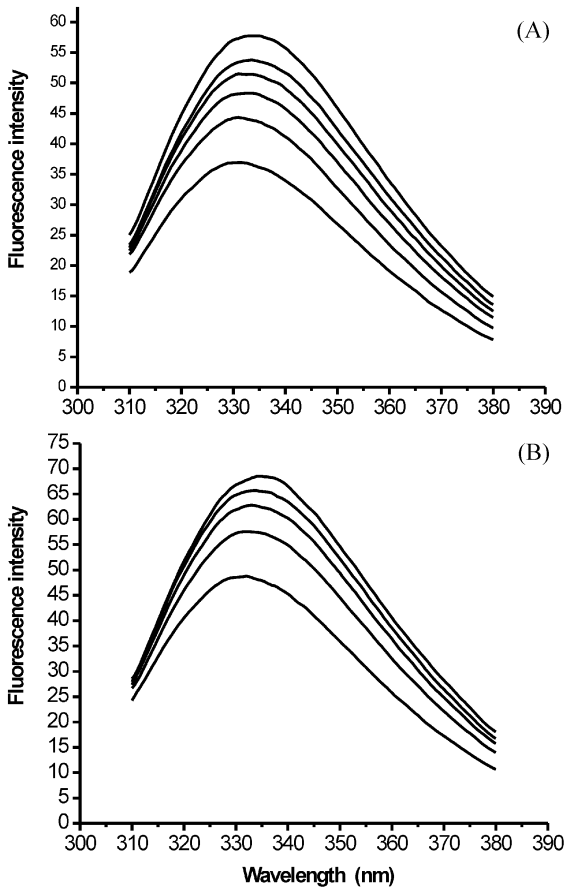

Fig. 7. (A) Intrinsic fluorescence emission spectra of AK (4.5 μM) in the presence of different zinc concentrations at 30°C. The zinc concentrations were 0 mM, 0.2 mM, 0.6 mM, 1.0 mM, 2.5 mM, and 3.5 mM from the bottom upward. (B) Intrinsic fluorescence emission spectra of CSI (4.5 μM) in the presence of different zinc concentrations at 30°C. The zinc concentrations were 0 mM, 0.2 mM, 0.6 mM, 1.2 mM, and 3.5 mM from the bottom upward.

different. Although a low zinc concentration causes hydrophobic exposure and the α-helical content of CK to increase, still there is no activity decrease (Tong *et al*., 2000), which is contrary to the result for AK. It was reported that 0.5 M urea can induce an increase of the AK α-helix content, accompanied with an increase of activity (France and Grossman, 1996). The reason was partially attributed to a change in polypeptide flexibility in the domain of the active site (France and Grossman, 1996). Similar results were also observed in our study. The increased activity, which may be related to the higher α-helix content, is probably a common phenomenon in the phosphagen kinase group that includes AK and CK. As far as CSI was concerned, CSI had lower activity at the same zinc concentration. The conformational changes, including hydrophobic exposure and the α-helical content decrease of CSI, were stronger than those of AK. These results suggest that although CSI under the effect of dilute urea has more α-helical content that is accompanied with higher activity than native AK, still more hydrophobic exposure when combined with more fragile active sites may make CSI's structure more susceptible to destruction at a low zinc concentration that is accompanied with lower activity.

However, with the further increase of zinc concentration, there was more hydrophobic exposure and a greater decrease of α-helical content. This was accompanied by decreased activity in AK and its CSI, which indicated that partial unfolding occurred. The change of α-helical content was greater than that of hydrophobic exposure and the intrinsic fluorescence maximum, which consisted with the higher conformational rate change in 222 nm ellipticity when compared with that in ANS fluorescence intensity (Table 2). Therefore, the α-helical structure of AK and its CSI were more sensitive to the effects of zinc. CSI changed more quickly in all of these cases. These results suggest that CSI is more sensitive to zinc concentration change. Thus, CSI is more unstable than native AK. Table 1 shows that this inactivation is a function of zinc ion concentration and is related to urea content. This suggests that the inactivation is at least a second order reaction. The k_1 and k_2 values of CSI are higher than that of AK, which may explain the faster inactivation rate for CSI that is induced by various zinc concentrations. Data from Table 2 might further prove that CSI is more flexible in structure and more easily affected by zinc.

It seems that the effects of Zn^{2+} on AK are dissimilar at different concentrations, which corresponds to the results from

Table 3. Comparison of activity and conformation of native AK and its CSI, and that of the two structures induced by zinc

	Native AK			CSI		
	Native AK	AK induced by 0.1 mM zinc	AK induced by 1 mM zinc	CSI	CSI induced by 0.1 mM zinc	CSI induced by 1 mM zinc
Activity (%)	100	77	10	108	63	4
ANS fluorescence maximum (nm)	464	463	463	465	464	461
ANS fluorescence intensity (%)	100	102	181	100	121	168
Intrinsic fluorescence maximum (nm)	331.5	331	331	332	333	333
Intrinsic fluorescence intensity (%)	100	110	149	100	109	129
222-nm ellipticty (m°)	-55.50	-54.09	-51.44	-59.16	-56.28	-52.01

the unfolding study of AK by guanidinium chloride (Gross *et al*., 1995). In the dilute denaturant, the effect of the denaturant may adjust the fine structure with little conformation change. However, great inhibition appears. As the denaturant concentration increases, there are great conformation changes and denaturation indeed occurs (Gross *et al*., 1995). Evidence shows that the interaction between AK and its charged substrates is predominantly electrostatic (Maurel *et al*., 1974). The pI of AK is about 6.3. The net negative charge on the surface, combined with its specific substrate binding mode, may make AK very sensitive to zinc ion and its concentration change, even at a dilute concentration. Many of these negatively-charged residues lie in, or nearby, the α-helix structure of AK (Zhou *et al.*, 1998); therefore, it is expected that the secondary structure changes have a close relation to the activity changes of AK and its CSI, and are more sensitive to the zinc concentration changes when compared with other conformational changes. Among these negatively-charged residues, the effects of zinc ion on Glu-314 and Asp-62 (Zhou *et al*., 1998) are possibly the most pivotal. These two residues are important in substrate binding or connecting two domains together to maintain the active conformation for catalysis; both lie on the surface of the protein molecule (Zhou *et al*., 1998). Therefore, in dilute denaturant, zinc's great influence on AK inactivation may be due to the effects of Zn^{2+} on the negatively-charged residues on the surface, especially the specific interaction of the positively-charged zinc ion with the negatively-charged carboxyl groups that are essential for catalysis. However, the undetectable conformational changes may come from its specific compact structure (35% α-helix content in AK (Zhou *et al*., 1998) *vs* 25% α-helix content in CK (Raimbault *et al*., 1997; Rao *et al*., 1998). Because there is a balance between the increased hydrophobic surface, which aids zinc ion binding, and electrostatic repulsion, which represses zinc ion binding, as the zinc concentration increases, then the effect of electrostatic attraction declines and the effect of structural stability begins to work. The inactivation of AK at high zinc concentrations is probably due to nonspecific Zn^{2+} binding to the inner surface of the enzyme; this binding may induce greater structural damage and more activity loss (Table 3). Nevertheless, it is also expected that AK, which has a compact structure that is combined with the balance between hydrophobic exposure and ionic forces, shows a slow decrease in residual activity and structural changes at a high zinc concentration (Table 3). The amino acid residues in the sequence surrounding the three tryptophans of AK are neutral or positively-charged (Zhou *et al*., 1998). This may explain the indistinct intrinsic fluorescence changes of both AK and its CSI. We proposed that the CSI's conformation decreases the stability of the global structure of AK and leads to the flexibility of regional parts around the key negatively-charged residues that may be on the surface of the protein. They can, therefore, be disturbed at zinc concentrations less than 0.1 mM, which were reflected by the activity and α-helix content changes.

As CK plays an important role in the cellular energy metabolism in vertebrates, any disturbance in the activity of CK may incur serious disease (Tong *et al.*, 2000). Therefore, CK research has attracted considerable attention, and the relationship between CK abnormalities in high zinc concentrations and Alzheimer's disease has been studied. However, little is known about AK that exists in invertebrates since it has not been investigated as extensively as CK. It has recently been discovered that *T. Cruzi*, a parasite which induces Chagas's disease, metabolizes arginine in mammalian tissues; however, AK is not present in host cells (Pereira *et al*., 2000). It is inevitable that zinc would affect the structure and function of AK and CK when the two enzymes work together in the same cellular environment. Also, the effects of zinc on AK and CK in a dilute zinc solvent are quite different. Therefore, further studies on the structures and functions of both enzymes and the definitive mechanism of zinc ion's effects on them will be helpful to find suitable chemotherapeuic agents against this kind of parasitic disease. These studies are important not only in theoretic research but also for future pathologic treatment.

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