

Multi-NMR and fluorescence spectra study the effects of aluminum(III) on coenzyme NADH in aqueous solutions

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Abstract

The interactions of dihydronicotinamide adenine dinucleotide (NADH) with Al(III) in near neutral aqueous solutions were studied by means of multinuclear (^{31}P , ^{27}Al , ^1H and ^{13}C)-NMR and fluorescence spectra techniques. The results suggested that Al(III) interacts with NADH by occupying the binding sites of pyrophosphate oxygen atoms and locks the adenine moiety of coenzyme in an *anti* folded conformation. Meanwhile, the weak attractive interactions ('association') may occur between Al(III) and the hydroxyl groups of ribose rings through the intramolecular hydrogen bonding. Furthermore, at biologically relevant pH and concentrations of Al(III) and NADH (pH 6.5, $C_{\text{Al}} = 10^{-6} - 10^{-5}$ M), Al(III) could increase the amount of folded forms of NADH, which will result in reducing the coenzyme NADH activity in hollow-dehydrogenases reaction systems. However, in the presence of possible competing organic acids such as citrate, oxalate and tartate, could detoxify these Al(III) toxic effect.

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1. Introduction

β -Nicotinamide adenine dinucleotide, NAD^+ , and its reduced form β -dihydronicotinamide adenine dinucleotide, NADH, act as coenzyme in enzymes that are ubiquitously involved in biological redox processes and play an important role in the conversion of chemical energy to useful metabolic energy [1]. The NAD^+/NADH linked de-

hydrogenases are a class of more than 150 enzymes in biological systems [2]. The NADH is composed of two 5'-nucleotides, AMP and dihydronicotinamide ribose 5'-phosphate, which are linked together by pyrophosphate bridge. The structure of NADH with its atom number scheme is shown in Fig. 1. There were many structure analysis including conformational studies of NADH in the past. Fluorescence spectroscopy [1], circular polarization [2], Raman spectroscopy [3,4], multinuclear (^1H , ^{13}C , ^{31}P)-NMR spectra [5–7] and X-ray crystallography [8–10] had been used to investigate the structural information. It was given that NADH two main limiting conformational forms

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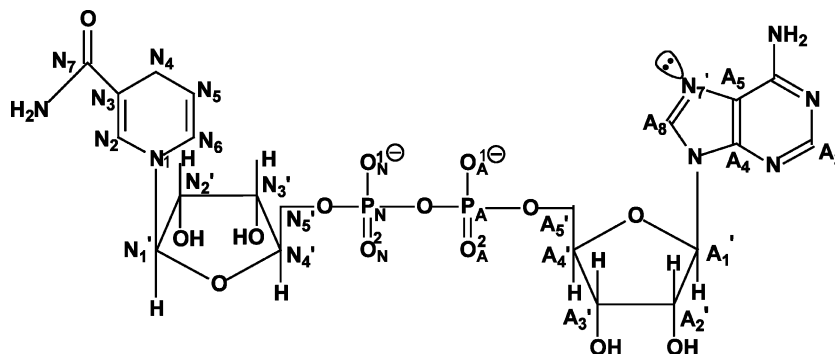


Fig. 1. Structure and atomic numbering of NADH.

existed in solution: extended/open form in which adenine and nicotinamide group lie about 10–12 Å apart and folded /stacked form in which the two base are stacked parallel to each other at about 3.4 Å apart. The stacked conformer can further adopt either a left- or a right-handed helical structure. Normally, in solution these conformational states are in rapid equilibrium with each other, and the amount of each depends on the conditions of pH, solvent and temperature. In these conformations, about 40% two of these are existed under physiological conditions [4]. More recently, Hoobler and coworkers utilized fluorescence excitation transfer spectroscopy to probe the conformational changes of NADH [1]. The results can be represented by using of two state models, and the mole fraction of aqueous NADH in the folder form at 295 K was determined to be 0.55, which is significantly higher than the previous report. However, the literature data on metal ions induced the conformational changes of NADH are rather scarce. There were only a few conformational studies of Mn^{2+} , Li^+ , La^{3+} and Eu^{3+} with NAD^+/NADH in the past [7,9–11], even though some metal ions have very important roles in the NADH participating enzymes reactions [12].

Al(III) ion is a highly cytotoxic metal to both plants and animals. It has been recognized as a major limiting factor of plant producing on acidic soils, which now account for about 40% of the earth's arable land and also responsible for a range of neurological disorders [13–17]. However, to date the cause of Al(III) toxicity has remained elusive and controversial. The mechanism of these

actions will be an essential area of aluminum chemistry in the future [18]. More recently, it has been proposed that some primary sites of toxicity may be associated with the insertion of Al(III) in metal binding domains of enzymes, causing a disruption in cell metabolism [12]. Mitochondria, as the basic energy/producing organelles of cells, may be easy targets for the toxic effects of metal ions, including Al(III) ion [13,17]. It is demonstrated that, among the enzymes that participate in and link to the mitochondria tricarboxylic acid cycle, Al(III) activates α -ketoglutarate dehydrogenase and succinate dehydrogenase. At the same time, aconitase and glutamate dehydrogenase (GDH) exhibit a decreased activity in the presence of this metal ion [19,20]. Accordingly, it is reasonable to assume that Al(III) could interfere with the bioenergetics of mitochondria, which is affected by degenerative disorders related to biology 'aging'. In addition, the inhibitory also affects the formation of glutamate in the transamination process, which may influence the amino acid synthesis and contribute to the Al(III) toxicity in higher plants [21]. Among these key metabolisms, NADH as a coenzyme plays an important role. Since the biology 'aging' is a transformation form between an energy rich state to an energy poor state, NADH can play an anti-aging role in the creation of new energy. Another anti-aging effect is due to the key role of NADH playing in cell regulation and in DNA repair. The more DNA that needs to be repaired, the more NADH will be used by the cell [22,23]. Therefore, it is very interesting to investigate of Al(III) interacts with coenzyme

NADH in order to further understand Al's role in the mechanism of dehydrogenase enzyme reaction and biology aging process. However, to date the study of interaction between Al(III) with NADH has been scarcely found in the available literature. Abou-Seif MAM has clarified that vanadium-mediated oxidation of NADH is enhanced by aluminum [33]. Accordingly, in this research, we have utilized ^1H , ^{13}C , ^{31}P and ^{27}Al -NMR and fluorescence spectroscopy to study the interactions of Al(III) with NADH in near neutral aqueous solutions. The conformational changes of this bimolecular induced by the metal ion are also discussed in order to understand the Al's role in biological enzyme catalytic reactions at a molecular level. Such bioinorganic studies are performed to clarify various aspects of structures of Al–NADH complexes, and to fill the gap between the knowledge concerning biochemical and biological phenomena.

2. Experimental and methods

2.1. Chemicals and reagents

β -Nicotinamide adenine dinucleotide, reduced form (β -NADH), disodium salt were purchased from Shanghai Lizhu Dongfeng Biotechnology Co. Ltd. (>90%), and used as received form. All NADH solutions were prepared fresh daily with double distilled water. Al^{3+} solutions were prepared by dissolving high purity metallic Al powder (99.99%) in hydrochloric acid. More dilute solutions were prepared by diluting this solution with double distilled water. Buffer solutions were prepared by mixing appropriate amount of Tris(hydroxymethyl)aminomethane with HCl (pH 6.5). Ethanol (EtOH), D_2O was used as received form Beijing Chemical Company. Most chemicals are of analytical reagent grade. ^1H -, ^{13}C -, ^{31}P - and ^{27}Al -NMR measurements were prepared by dissolving appropriate amount of NADH and $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ in D_2O and buffer solutions. Necessary polyethylene vessels were used. All glass-wares were soaked in 10% HNO_3 for at least 24 h, then washed carefully with double distilled water. The pH values in D_2O solutions were corrected for the

deuterium isotope effect by adding 0.45 to the meter reading [24].

2.2. Apparatus and methods

The ^{31}P - and ^{27}Al -NMR spectra were obtained on the Bruker DRX500 spectrometer (Swiss) at 201.5 and 130.3 MHz, respectively. Chemical shifts were referenced to an external coaxial insert containing 0.1 M H_3PO_4 (0 ppm) or 0.1 M $\text{Al}(\text{H}_2\text{O})_6^{3+}$ (0 ppm) placed with every sample for ^{31}P -, ^{27}Al -NMR experiments. The ^1H -, ^{13}C -NMR were performed on a Bruker AVANCE 300 spectrometer at 300.1, 75.5 MHz, respectively, and 10000 scans were accumulated per ^{13}C spectrum. Chemical shifts were referenced to D_2O (4.70 ppm) or TMS (0 ppm) for ^1H -, ^{13}C -NMR experiments. All NMR measurements were carried out at ambient temperature (23 °C). The pH value of solution was measured with 77785 pH-metric Radiometer (Denmark). Fluorescence experiments were performed on a model RF-5301 of spectrofluorimeter (Shimadzu, Japan). The measurements were carried out at room temperature. Excitation and emission wavelengths were 260 and 460 nm, respectively. And both spectra for the solvents and reactants were obtained to correct for background fluorescence of the EtOH, Al(III) and buffer solutions. These experiments were carried out at pH 6.5 with Tris–HCl buffer solutions since this value was representative of the cytoplasmic pH in plants roots [25].

3. Results and discussion

3.1. Multi-NMR spectra studies

In order to examine the complexation and conformation of the Al–NADH systems, the extensive ^{31}P -, ^{27}Al -, ^1H - and ^{13}C -NMR measurements were carried out in near neutral aqueous solutions. The effect of Al(III) on the ^{31}P -NMR spectra of phosphate ligands depends primarily on the number of phosphates. When Al(III) binds to diphosphates, it is always accompanied by broadening and upfield shifting, and we therefore considered such shifts as indicative of direct

binding of Al^{3+} ion to phosphate oxygen atoms [32]. Because of the acidic decomposition at $\text{pH} < 5.0$ and high pH precipitation of Al-NADH , only one Al-NADH spectrum at $\text{pD} 6.0$ was obtained. For free ligand of NADH, except for external coaxial insertion $0.1 \text{ M H}_3\text{PO}_4$ peak at 0 ppm , one peak ($\delta = -10.6 \text{ ppm}$) appeared in the spectrum (Fig. 2A). While for Al-NADH complexes (0.02 M NADH , $\text{Al-NADH} = 1:2$, $\text{pD} 6.0$ Tris-DCI buffer), it showed four resonances in the system: -10.5 , -11.5 , -14.6 and -20.7 ppm (Fig. 2B). Among these peaks, -10.5 ppm represented the free ligand NADH pyrophosphate oxygen atom

resonance in its deprotonation form [6,7]. The other peaks can be attributed to three different species with different binding modes and structures. Meanwhile, the ^{27}Al -NMR spectra of NADH and Al-NADH (0.02 M NADH , $\text{Al-NADH} = 1:2$, $\text{pD} 6.0$ Tris-DCI buffer) are shown in Fig. 2C and D. Examination of the spectrum of Al-NADH solution, we observed four peaks, one for the reference aqua complex $\text{Al}(\text{H}_2\text{O})_6^{3+}$ around 0 ppm , and the others are probably attributed to three different species resonances: $\delta = -3.21 \text{ ppm}$, $\delta = -6.95 \text{ ppm}$ and one at around $\delta \leq 0 \text{ ppm}$, respectively. Therefore, the three different complexes with different structures and consequently different spectra existed in the solution. According to the above ^{31}P -, ^{27}Al -NMR spectra, it is concluded that: (1) Al(III) can interact with coenzyme NADH through the diphosphate oxygen (O_N^1 , O_A^1) to form Al-NADH species in near neutral Tris-DCI buffer solutions; (2) since the NADH in the interaction is potentially multi-dentate and a variety of stoichiometries are possible, three kinds of different Al-NADH species may exist in aqueous solutions. And the exact structures of these species need to be further studied.

We applied ^1H -NMR technique to study the interactions of Al(III) with NADH in near neutral aqueous solution. The effects of Al(III) on the ^1H -NMR spectra of NADH are demonstrated in Fig. 3. The ^1H -NMR spectrum of free ligand NADH in D_2O without buffer solution at $\text{pD} 5.0$ is shown in Fig. 3A. Except for NADH, there is few foreign matter EtOH in the component. The assignment of NADH signals was referenced to the literature [7]. The ligand NADH and the complexes Al-NADH are at the same concentration and the same pH value (0.02 M NADH , $\text{Al-NADH} = 1:2$, $\text{pD} 6.0$) with buffer solution of Tris-DCI. Comparing to ligand spectrum (Fig. 3B), resonances signals from the A_8 , H-A_2 , $\text{H-A}'_1$, $\text{H-N}'_1$, H-N_2 and the H signals of the ribose rings were broadened in the presence of Al(III) . However, these peaks did not upfield or downfield shifted (Fig. 3C). Especially, a much bigger broadening effect was observed for the signal of H-A_8 than that of H-A_2 . It implied that Al(III) bound with NADH in the pyrophosphate oxygen (O_A^1 , O_N^1) in the complex [7], which

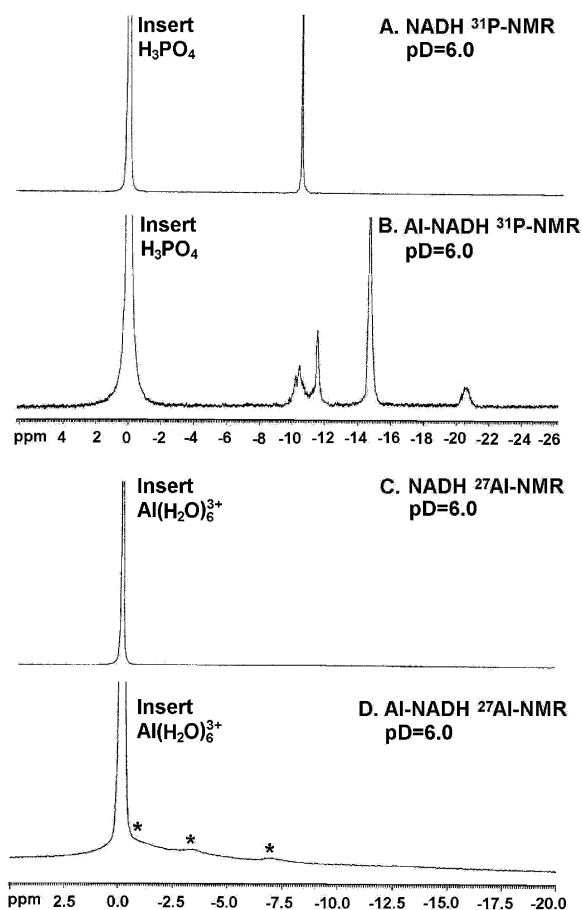


Fig. 2. (A) ^{31}P -NMR spectra of NADH (0.02 M), $\text{pD} 6.0$, Tris-DCI buffer; (B) ^{31}P -NMR spectra of Al-NADH ($0.01\text{--}0.02 \text{ M}$), $\text{pD} 6.0$, Tris-DCI buffer; (C) ^{27}Al -NMR spectra of NADH (0.02 M), $\text{pD} 6.0$, Tris-DCI buffer; (D) ^{27}Al -NMR spectra of Al-NADH ($0.01\text{--}0.02 \text{ M}$), $\text{pD} 6.0$, Tris-DCI buffer.

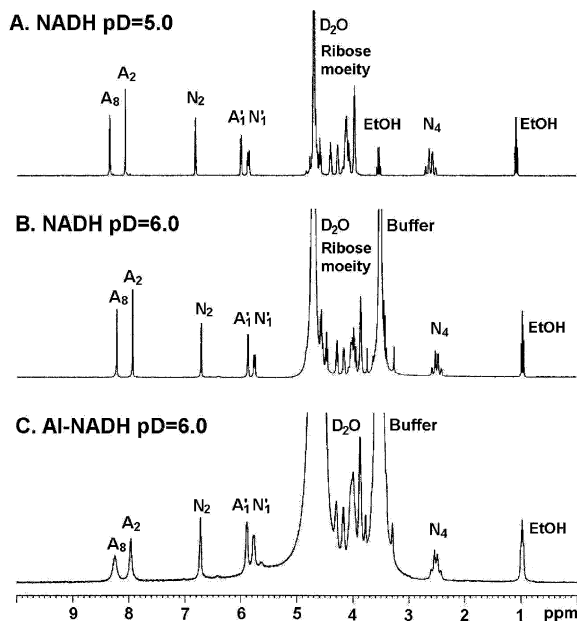


Fig. 3. ^1H -NMR spectra of NADH and its Al(III) complexes. (A) NADH, 0.02 M; pD 5.0; (B) NADH, 0.02 M; pD 6.0, Tris-DCI buffer; (C) Al-NADH, 0.01–0.02 M; pD 6.0, Tris-DCI buffer.

is in accordance with above ^{31}P -NMR result. Since the adenine H-A₈ of NADH is juxtaposed to the pyrophosphate backbone in the *anti* form whereas adenine H-A₂ is juxtaposed in the *syn* form [7]. Accordingly, if Al(III) binds to the pyrophosphate backbone of pyridine coenzyme, one would expect that the metal ion to broaden or shift H-A₈ or H-A₂ depending upon whether the conformation is *anti* or *syn*. Therefore, the ^1H -NMR spectra proved that the adenine moiety of NADH existed in an *anti* folded conformation structure [7]. In addition, the broadened hydrogen signals of ribose sugar rings may suggest the existence of weak attractive interactions ('association') between Al(III) ion and the hydroxyls of ribose rings through the intramolecular hydrogen bonding [26]. The impurity EtOH of NADH did not shift in the presence of Al(III) ion indicated that EtOH does not influence the interactions of Al and NADH in this condition. This point was further confirmed by the following ^{13}C -NMR spectra.

The ^{13}C -NMR spectrum of free ligand NADH in D₂O without buffer solution at pD 5.0 is shown

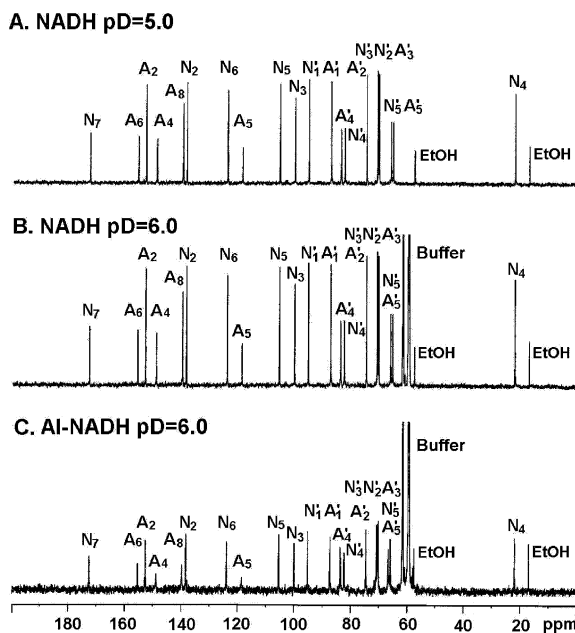


Fig. 4. ^{13}C -NMR spectra of NADH and its Al(III) complexes. (A) NADH, 0.02 M; pD 5.0; (B) NADH, 0.02 M; pD 6.0, Tris-DCI buffer; (C) Al-NADH, 0.01–0.02 M; pD 6.0, Tris-DCI buffer.

in Fig. 4A. Like above ^1H -NMR spectrum, there is little impurity EtOH in the reagent. The assignment of NADH carbon signals was referenced to the literature [5]. Meanwhile, the spectra of NADH and Al-NADH did not show significant change for the signals of dihydronicotinamide after mixing. It suggested that Al(III) does not interact with these groups (Fig. 4C). However, comparing to ligand spectrum (Fig. 4B), the following information can be obtained from the Al-NADH spectrum (Fig. 4C): (1) the slightly broadened and downfield shifted signals appeared for the C-A_{5'}, C-N_{5'} in the complexes, which are closed to pyrophosphate oxygen atoms, indicated that the deprotonated pyrophosphate oxygen (O_A¹, O_N¹) are the preferential binding sites of Al(III) with NADH; (2) the little broadened but not obviously shifted signals of C-A₈, C-A₄ and C-A₅ in the complex spectrum, also reflected Al-NADH adenine moiety tends to form a *anti* folded conformation [7]; (3) the broadened signals of C-A_{2'}, C-A_{3'} and C-N_{2'}, C-N_{3'} revealed the existence of attractive interactions ('association') between Al(III) ion and

NADH hydroxyls of ribose rings through intramolecular hydrogen bonding [26]; (4) the almost unchanged carbon signals of foreign matter EtOH of NADH in the presence of Al(III) confirmed that EtOH does not influence the interactions of Al–NADH in this case. These results well agreed with the previous ^1H -NMR spectra study. Furthermore, another fact that nicotinamide N_4 resonance in the ^1H -, ^{13}C -NMR spectra of Al–NADH almost did not change implies that Al(III) did not influence the carboxamide group of nicotinamide in this pH region and also did not promote the oxidation process of the NADH at this condition.

3.2. Fluorescence spectra studies

In above complexation and conformational studies, Al(III) concentration is at 10 mM, which is irrelevant to physiological condition. Accordingly, it is necessary to study the interaction of Al(III) with NADH, at lower Al(III) concentration and especially in the plant cell physiologically more relevant concentration range (μM) and near neutral pH range. Comparable energy transfer between molecules would require concentrations on the order of 10^{-2} M, working at 10^{-5} – 10^{-6} M allows observation of only intramolecular energy transfer with the observed fluorescence dependent on the separation between the adenine and dihydronicotinamide nucleotides [1]. The two chromophores of NADH, adenine and dihydronicotinamide nucleotides, have absorption bands at approximately 260 and 340 nm, respectively. In this work, the adenine nucleotide was excited at 260 nm; emission from the dihydronicotinamide nucleotide and was monitored at around 460 nm. A sample of experimental data showing the changes in fluorescence intensity as a function of Al(III) concentration is presented in Fig. 5A in H_2O solution and B in H_2O –EtOH (1:1) mixture solution. As shown in Fig. 5A, the increase of the amount of Al(III) in NADH aqueous solution resulted in a substantial increase in NADH fluorescence intensity of the peak of wavelength. This weak increase can be attributed to the development of NADH folded form conformation, which is only available for the probe of

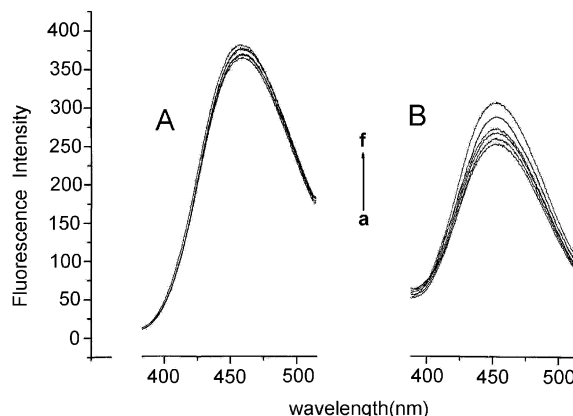


Fig. 5. Fluorescence spectra showing the increase in emission intensity of Al–NADH system as the Al(III) concentration increases. Excitation wavelength is 260 nm. $C_{\text{NADH}} = 10 \mu\text{M}$, in 50 mM pH 6.5 Tris–HCl buffer: (A) in H_2O solution; (B) in H_2O –EtOH (1:1) solution. L/Al ratios: (a) 1:0; (b) 1:1/4; (c) 1:1/2; (d) 1:1; (e) 1:2; (f) 1:4, respectively.

folded form of NADH efficient fluorescence energy transfer [1]. At the same time, we tested the same Al–NADH complex system in the H_2O –EtOH (1:1) solution as shown in Fig. 5B, the whole complex system fluorescence intensity were decreased as a result of intramolecular stacking of dinucleotides is solvent-dependent, with increasing apolarity favoring the unstacked state, which is in agreement with the earlier finding [1]. However, the obvious increases of fluorescence intensities as a function of the increased ratio of Al/NADH is ascribed to the enhanced partitioning of the folded-form probe, which is resulted from the interaction of Al(III) with NADH. According to the experimental data, the plot of fluorescence intensity versus the ratio of the concentration of Al(III) to NADH is given in Fig. 6. The complex ratio of Al(III) to NADH is supposed to 1:1. It indicated the bimolecular NADH bound to Al(III) under these plant cell physiological pH and Al(III) concentration conditions.

At the near-neutral pH of the cytoplasm, most organic acids existed in the deprotonation form. Among these, citrate, oxalate and tartrate are some of the commonly released organic acid anions that can form sufficiently strong complexes with Al^{3+} to protect plant roots [14–16]. Moreover, upon formation of the Al–CaM (calmodulin) complex,

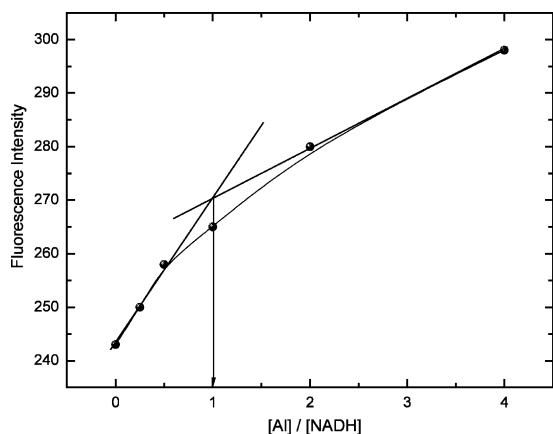


Fig. 6. The dependence of fluorescence intensity on $C_{\text{Al}}/C_{\text{NADH}}$ in H_2O – EtOH (1:1) solution. $C_{\text{NADH}} = 10 \mu\text{M}$, in 50 mM pH 6.5 Tris–HCl buffer.

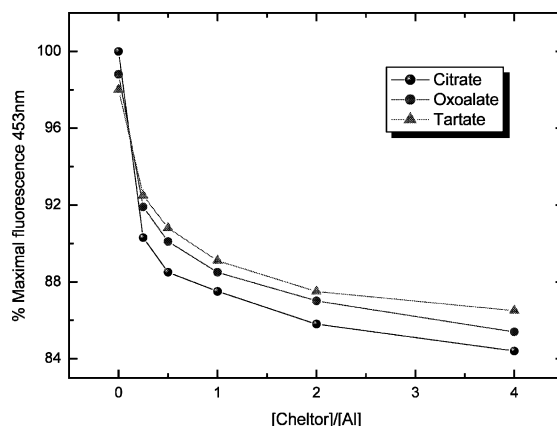


Fig. 7. Decrease of aluminum-induced folded forms of NADH, with the increase of organic acids as Tris–HCl buffer, pH 6.5. The 100% values of the NADH fluorescence intensity are corresponded to that characteristic for a molar ratio of 2:1 for $C_{\text{Al}}/C_{\text{NADH}}$.

organic acids were able to partially reverse the Al-induced conformational change of the protein [25]. Since the organic acids cannot develop the fluorescence intensity of NADH, the effects of organic acids effectively prevent and decrease in the Al-triggered NADH fluorescence intensity are ascribed to the subsequent increase of the interactions of Al(III) with organic acids in aqueous EtOH mixtures (Table 1). And as shown in Fig. 7, it also clearly indicated these phenomena. As a result, despite of the formation of the Al–NADH complexes, organic acids are still able to reverse the enhanced fluorescence intensities, which are associated with conformational changes of the NADH. The efficiency of this reversal follows

the sequence: citrate > oxalate > tarate, which is in agreement with the values from earlier findings of Al–CaM [25]. Coordination of Al(III) to these organic acids in near neutral pH solutions strongly occurs through the carboxyl and the hydroxyl groups according to the previous reports [27,28].

As a result, according to the multinuclear (^1H , ^{13}C , ^{31}P and ^{27}Al)-NMR studies of 0.02 M NADH and Al–NADH (1:2), in the near neutral state and at physiological pH, the pyrophosphate group of NADH bears two negative charges distributed over free oxygen atoms, which are facilitated to bind with Al(III). These bindings of Al(III) with

Table 1

Effects of organic acids on the fluorescence intensity of NADH in the presence and absence of Al(III), the molar ratio of 2:1 for $[\text{Al}]/[\text{NADH}]$, in the pH 6.5 Tris–HCl buffer aqueous EtOH (1:1) solutions

Organic acid [Chelator]	[Al]	[Chelator]/[NADH]					
		0:1	1/4:1	1/2:1	1:1	2:1	4:1
Citrate	–	252.0	255.5	254.1	252.1	251.7	254.3
Oxalate	–	253.2	255.6	253.8	252.0	253.1	252.2
Tartate	–	252.1	252.8	254.3	253.4	254.4	255.0
Citrate	$2 \times 10^{-5} \text{ M}$	296.3	267.5	262.1	259.2	256.3	250.1
Oxalate	$2 \times 10^{-5} \text{ M}$	292.8	272.4	267.0	262.1	259.6	252.9
Tartate	$2 \times 10^{-5} \text{ M}$	290.4	274.3	269.0	264.1	259.2	256.3

NADH are explained best the adenine moiety of NADH is locked in the *anti* conformation (or the dihydronicotinamide moiety of NADH is locked in the *syn* conformation). And it is possible that the intramolecularly folded conformation of the dinucleotide existed in this pH region [7]. Meanwhile, some OH groups of the sugar unit could form weak hydrogen binding with water molecules or ions OH^- in the Al(III) coordination sphere [26,29]. At the near-neutral pH of the cytoplasm and physiologically more relevant concentration range (μM) of Al(III), the fluorescence spectra showed there were only increased intensities, not shifted peaks in Fig. 5. This result indicated Al(III) coordinated with two exposed pyrophosphate oxygen atoms, instead of complex with the stacking adenine N_7 in this condition. Also this conclusion agreed with above multinuclear NMR spectra findings. Since from the stereo-chemical considerations, a 'folded', helical structure for NAD^+/NADH is feasible only if no chelation exists between adenine N_7 and O_N^2 of the P_N phosphate group [9,10]. Moreover, it is very likely that Al(III)-hydroxo species in some metastable state (which is fairly common in aqueous Al(III) solutions, especially in a diluted solution) form outer-sphere Al(III) complexes through H_2O or OH^- hydrogen bonding with the hydrated NADH moieties [26,29]. In this case, the folded form of NADH may be fixed, which resulted in increasing the fluorescence intensity. In other words, Al–NADH complexes or Al(III)-hydroxo species hydrogen bonding probably stabilizes a fairly well defined conformation with a certain amount of folded structure. When we changed the solvent by using H_2O –EtOH (1:1) solution from H_2O , Al(III) might have higher binding ability to NADH in H_2O –EtOH solution than that of only in H_2O solution. Owing that Al(III) ion has weaker hydrolytic ability in H_2O –EtOH solution, and therefore, has more aggressive interaction ability in the apolarity solution. The organic acids revised ability of the enhanced fluorescence intensities of Al–NADH not only proved the organic acids detoxified Al(III) effects, but also contrarily confirmed these enhanced fluorescence intensities of NADH are caused by Al(III).

4. Conclusion

Although the effects of Al(III) on biological systems have been extensively described, direct information concerning the molecular basis of its effects on enzyme systems and cell culture is rather scant [17]. Al(III) influences (inhibits or activates) not only acid–base enzymes, but also some redox enzymes (including dehydrogenase) [12]. A number of dehydrogenases with and without bound NAD^+/NADH have been crystallized and subjected to X-ray analysis. These studies invariably demonstrated that NAD^+/NADH exist in an 'extended' form, with sugar and pyrophosphate moieties bound by amino acid side chains while the adenosine heterocycle is inserted into a hydrophobic pocket the protein [9,10,30]. Since most dehydrogenase are either activated by some metal ions (Mg^{2+} , Ca^{2+}) or use nucleotides (ATP/ADP, NAD/NADH) as a cofactor, the effect of Al(III) in these enzymatic processes is generally the displacement of metal ions or its interaction with the nucleotides, through their phosphate functions. The above results suggested the occurrence of an Al(III)-linked conformational changes in these flexible molecules, which brings structural changes at the primary (by occupying the binding sites of phosphate oxygen O_N^1 , O_A^1 and hydroxyls of ribose rings) and secondary (restrict folded form of NADH in plant cell physiological pH and Al(III) concentration) recognition sites for substrates and enzyme. These effects could decrease the NADH activity in the enzyme reactions. Because GDH represents a key enzymatic link between catabolic and biosynthetic pathways, and is ubiquitous in both higher and lower organisms [31], therefore these effects may result in influence the coenzyme NADH activities in holodehydrogenase reaction systems. The presence of possible competing ligands such as citrate, oxalate and tartate, could detoxify these Al(III) toxic effects. This organic acids detoxification effect was in good agreement with the previous reports [14,15,25]. Concerning the organic acids' protective role, our physico-chemical findings are consistent with those derived from physiological experiments. Consequently, our conclusion that organic acids protect the

coenzymes NADH from Al(III) lesions is of a biological relevant value in this respect.

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