Spectrochimica Acta Part A 78 (2011) 1305–1309

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Spectroscopy study on the noncovalent interactions in the binary and ternary systems of L-lysine, adenosine 5′-triphosphate and magnesium ions

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ARTICLE INFO

Article history:
Received 26 September 2010
Received in revised form
28 December 2010
Accepted 6 January 2011

Keywords:
ATP
Lysine
Magnesium ion
Noncovalent interaction
Recognition

ABSTRACT

Intermolecular interactions of adenosine 5′-triphosphate (ATP) with Lysine (Lys) and Mg^{2+} were studied in aqueous solution by ^{1}H and ^{31}P NMR spectra. In the metal-free system, the N-1 atom of the purine ring of ATP and carboxyl group of Lys are the interaction sites at low pH conditions. With increasing pH, the interaction efficiency between the phosphate group of ATP and the protonated ammonium group of Lys increased significantly, while that with carboxyl group in Lys decreased. In the Mg^{2+}–Lys–ATP system, multi-interactions, such as coordination, cations (Mg^{2+}, NH_3^+)–π, hydrogen bonding, ion-pairing interactions and electrostatic interactions co-existed. In addition, the recognition of ATP by the amino acid cation (Lys) was significantly promoted by the addition of magnesium ion, which led to the coordination competition between Lys and ATP.

1. Introduction

Enzyme catalyzed reactions with adenosine 5′-triphosphate involve divalent metal ions, usually Mg^{2+} [1–3]. Therefore, understanding the interactions of ATP with protons and metal ions in catalyzed phosphoryl or nucleotydyl transfer processes is of great importance. In recent years, there have been considerable works in investigating the molecular mechanisms of the binary metal complexes formed with ATP. In the case of binary complexes with ATP, metal ions are bound with ATP *via* both oxygen donors of the phosphate group and the nitrogen atoms of the purine nucleus [1,4–6]. The triphosphate group and the adenine ring are also the sites of noncovalent interactions with the other bioligands, such as polyamines or amino acids. Natural polyamine and synthetic ligands, for example macrocyclic polyammonium, phenanthroline, and polyoxomolybdates, have been developed to elucidate the features of the interactions involving ATP and metal ions [7–11]. The ligands bind strongly and selectively to ATP by electrostatic interactions and hydrogen bonds between the cationic binding sites of the receptor and the negatively charged phosphate groups of the substrate when they are protonated in the aqueous solution. However, there are only a few publications focused on the interactions in the ATP–amino acid binary complexes and the ATP–amino acid–metal ion ternary complexes [12–16]. Structural modifications or mutation of any of the amino acids may affect functions of the living cells, e.g., enzyme loses the catalytic activity when the key amino acids are chemical modified or mutated to other amino acids [17,18]. Metal ions in the living organisms modify the characters of bioprocesses. Hence, an understanding of various interactions involving ATP, amino acids, and metal ions at the molecular level is one of key problems in understanding their functions, especially the interactions of amino acid/ATP or amino acid/ATP/metal ion in a complex system.

L-lysine, one of cationic amino acids, is a key amino acid residue which takes part in the energy conversion of ATP in adenylate kinase or ATPase [17–20] because the protonated side chain in Lys can interact with the oxygen atoms of the phosphate chain in ATP. Therefore, understanding the mechanisms for interactions between ATP, Lys and metal ion is helpful in elucidating the role of the ATP binding pocket [13,14]. However, studies on the interactions between Lys and ATP, or the interactions in the ternary system in the presence of metal ions are few. This paper focused on the interactions of ATP with Lys in the absence and in the presence of Mg^{2+}. The results based on NMR provide further clue to elucidate the function of Lys residue in ATP conversion.

2. Materials and methods

Adenosine 5′-triphosphate disodium salt (99%), adenosine-5′-diphosphate (ADP, 99%), adenosine-5′-monophosphate (AMP, 99.7%), and L(+)-lysine (99%) were purchased from Acros Organics (USA). Their structures are shown in Fig. 1. $MgCl_2{\cdot}6H_2O$ was

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doi:10.1016/j.saa.2011.01.001

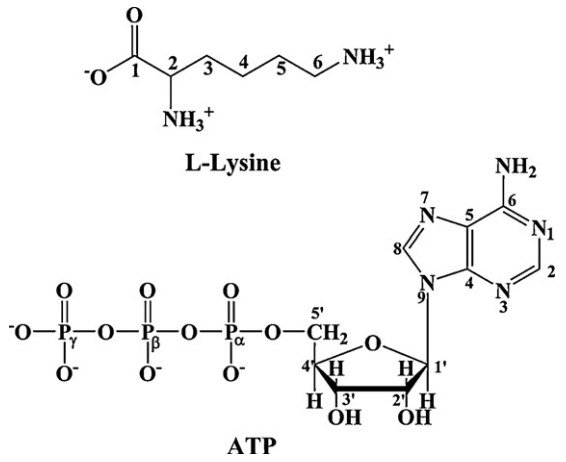

Fig. 1. The structures of the bioligands.

purchased from Beijing Shuanghuan Chemical Reagent Co., Ltd. (China). Deuterium oxide (D_2O, 99.9%) was obtained from Cambridge Isotope Laboratories Inc. All of these chemicals, except $MgCl_2{\cdot}6H_2O$, were used without further purification.

Fluorescence spectra were measured with a F-4500 Spectrofluorophotometer (Hitachi, Japan), using 5/5 nm slit widths. The excitation wavelength was 281 nm, and the emission spectra were recorded in the 300–550 nm range. 3.0 mL of solution containing an appropriate concentration of ATP, ADP, and AMP was titrated by successive additions of lysine to generate a final concentration of 6.0×10^{-6}–2.0×10^{-5} mol L^{-1} of lysine.

^1H and ^{31}P NMR spectra were obtained on an Inova-400 MHz spectrometer. In the ^{31}P NMR spectra, the chemical shifts were relative to an external reference of 85% H_3PO_4. pH was adjusted by adding tiny drops of NaOH or HCl solution, and was determined by a Markson 6200 pH meter equipped with a glass combination pH electrode. The standard aqueous buffer of pH 4.00 and 7.00 were used to calibrate the pH meter at 293.15 K. The solutions of ATP, with and without Lys, or $MgCl_2$, were prepared by quantitatively dissolving the chemical(s) in twice-distilled H_2O with 30% D_2O for field lock purpose. The molar ratio of ATP–Lys was 1:1, and that of the ternary system was also 1:1:1. The concentration of the samples for all experiments was 0.1 mol L^{-1}.

3. Results and discussion

3.1. Lys–ATP binary system

3.1.1. Fluorescence

Fig. 2 shows the fluorescence emission spectra of ATP in the absence and in the presence of Lys. Obviously, ATP displays an emission maximum at 385 nm when the excitation wavelength is 281 nm. Lys has no intrinsic fluorescence, however, since it lacks a conjugated system or an aromatic moiety. In Fig. 2, the addition of Lys resulted in significant decrease of the fluorescence intensity of ATP, as well as conspicuous change in the emission spectra. The fluorescence intensity of ATP decreases with increasing Lys concentration, which is compared with the fluorescence intensity of ATP in the absence of Lys. It clearly indicates that the interaction exists between Lys and ATP in aqueous solution. Lys has a cationic side chain and another ammonium group, which can interact with the phosphate oxygen atoms or the N-1 or N-7 site of the adenine ring of ATP by hydrogen bonds and electrostatic interactions. The fluorescence intensity of nucleotide was found to be dependent on the number of phosphate groups in the nucleotide molecule [21]. Thus, the fluorescence intensity of ATP decreased when Lys was added

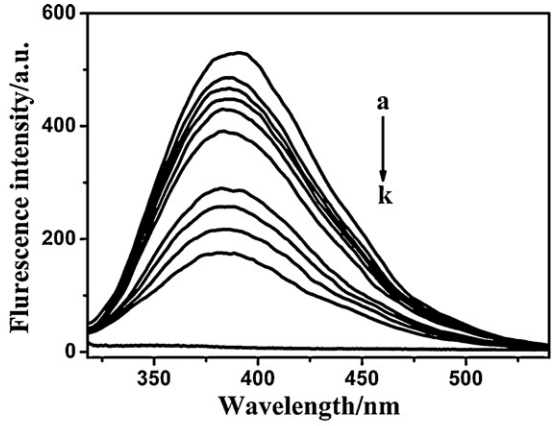

Fig. 2. The fluorescence emission spectra of ATP–Lys system: (a) 1.0×10^{-4} mol L^{-1} ATP; (b–j) 1.0×10^{-4} mol L^{-1} ATP with 6.0×10^{-6} mol L^{-1}, 7.0×10^{-6} mol L^{-1}, 8.0×10^{-6} mol L^{-1}, 1.0×10^{-5} mol L^{-1}, 1.3×10^{-5} mol L^{-1}, 1.4×10^{-5} mol L^{-1}, 1.6×10^{-5} mol L^{-1}, 1.8×10^{-5} mol L^{-1}, 2.0×10^{-5} mol L^{-1} Lys; (k) 6.0×10^{-6} mol L^{-1} Lys only.

into the ATP solution, indicating the interaction between Lys and the phosphate groups of ATP in aqueous solution.

To better understand the interactions between nucleic acids and Lys, variations of relative fluorescence intensity in the Lys with ATP, Lys with ADP, and Lys with AMP systems were also investigated (Fig. 3). With increasing concentration of Lys, descending tendency of the fluorescence intensity of ATP is the most evident one. However, the fluorescence intensity of ADP decreases less in the ADP case than that in the ATP case, while the fluorescence intensity of AMP even has no change in the AMP solution. These findings suggest that Lys interacts with both γ- and β-phosphate while does not recognize the α-phosphate of ATP, which are also proofed by ^{31}P NMR spectra.

3.1.2. NMR

Interaction between Lys and ATP in aqueous solution was detected by ^1H and ^{31}P NMR spectra. Before the addition of Lys into the ATP solution, the ^1H chemical shifts change in the ATP solution as a function of pH is in agreement with the study by Wang et al. [22]. Fig. 4 shows the normal proton NMR spectrum of the adenine ring of ATP in the ATP solution, which serves as the reference for other spectra. The two peaks at 8.061 and 8.366 ppm are assigned to H-2 and H-8 protons at pH 7.0 in the ATP solution, which are in close proximity to the N-1 and N-7 atom of the adenine ring of

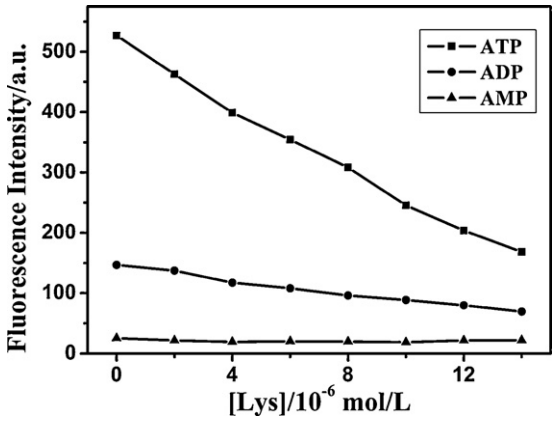

Fig. 3. Relative fluorescence intensity of ATP (1.0×10^{-4} mol L^{-1}), ADP (1.0×10^{-4} mol L^{-1}), AMP (1.0×10^{-4} mol L^{-1}) obtained by titration with Lys. [Lys] from 0 to 1.4×10^{-5} mol L^{-1}.