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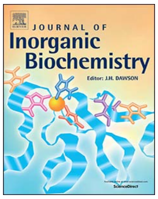

Copper(II) ions interactions in the systems with triamines and ATP. Potentiometric and spectroscopic studies

S.K. Hoffmann[a,*], J. Goslar[a], R. Bregier-Jarzebowska[b,*], A. Gasowska[b], A. Zalewska[b], L. Lomozik[b]

[a] Institute of Molecular Physics, Polish Academy of Sciences, Mariana Smoluchowskiego 17, 60179 Poznan, Poland
[b] Faculty of Chemistry, A. Mickiewicz University, Umultowska 89b, 61614 Poznan, Poland

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ABSTRACT

The mode of interaction and thermodynamic stability of complexes formed in binary and ternary Cu(II)/ATP/triamines systems were studied using potentiometric and spectroscopic (NMR, EPR, UV–Vis) methods. It was found that in binary metal-free systems ATP/H_xPA species are formed (PA: Spd = spermidine or 3,3-tri = 1,7-diamino-4-azaheptane) where the phosphate groups from nucleotides are preferred negative centers and protonated amine groups of amines are positive centers of reaction. In the ternary systems Cu/ATP/H_x(PA) as well as Cu/(ATP)(PA) species are formed. The type of the formed Cu(II) complexes depends on pH of the solution. For a low pH value the complexation appears between Cu(II) and ATP molecules via oxygen atoms of phosphate groups. For a very high pH value, where ATP is hydrolyzed, the Cu(II) ions are bound to the nitrogen atoms of polyamine molecules. We did not detect any direct coordination of the N7 nitrogen atom of adenosine to Cu(II) ions. It means that the Cu—N7 interaction is an indirect type and can be due to noncovalent interplay including water molecule. EPR studies were performed at glassy state (77 K) after a fast freezing both for binary and ternary systems. The glassy state EPR spectra do not reflect species identified in titration studies indicating significant effect of rapid temperature decrease on equilibrium of Cu(II) complexes. We propose the molecular structure of all the studied complexes at the glassy state deduced from EPR and optical spectroscopy results.

1. Introduction

Aliphatic polyamines (PA) - biogenic amines: putrescine (Put) = $NH_2(CH_2)_4NH_2$, spermidine (Spd) = $NH_2(CH_2)_3NH(CH_2)_4NH_2$ and spermine (Spm) = $NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$ occur in relatively large concentrations in cells of all living organisms [1–4] and take part in many biochemical processes [1,3,5]. Polyamines, compounds of high basicity, react in the form of polycations with negative fragments of such important biomolecules as DNA, RNA and protein, which affects the cell growth and the proliferation process. The presence of polyamines leads to changes in the structure of nucleic acids on several levels of their organization, which determines gene expression processes and genetic information transfer. The inhibition of the synthesis of polyamines in cells results in the retardation of proliferation [6,7].

Ribosomes with PA deficiency are less active compared to those with normal amine concentration, as well as unstable even at the appropriate level of magnesium [8,9]. The concentration of amines in the organism depends on the kind of tissue and cell age. A relatively high polyamine concentration, equal to about 2 mM spermidine or 4 mM spermine has been found in the pancreas [10]. The PA level varies even in different parts of the same organ. For instance, an average concentration of polyamines in human leukocytes is over a dozen times higher than in erythrocytes [11]. Of particular importance is the information that PA level increases in cancerous cells, which enables to monitor the development of illness and potentially paves the way for the search of chemotherapeutic drugs [12]. Polyamines also take part in the development of Alzheimer's disease and other transmissible diseases [13]. Despite research conducted for many years, we are still far from unequivocal determination of the PA role in the processes occurring in the organisms [14]. For example, a clearer elucidation is required which of the factors: polycation charge or its structure, plays the crucial role in noncovalent interactions in the systems of living organisms, taking into account the reactions of complex formations with metal ions present in the living cells.

Among the first convincing reports on the formation of molecular complexes as a result of noncovalent interactions involving protonated polyamines are those published almost 40 years ago [15,16]. Manning's polyelectrolyte theory suggests that the charge of reactants exerts the main effect on the mode of the interactions [17,18]. However, such an

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[*] Corresponding authors.
E-mail addresses: skh@ifmpan.poznan.pl (S.K. Hoffmann), bregier@amu.edu.pl (R. Bregier-Jarzebowska).

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approach does not explain the specificity of some reactions. Polyamines cannot be regarded as a point charge as it was assumed in the description of reactions of these biomolecules with metal ions, e.g. with magnesium [19]. The structure and spatial matching between polyamine and another bioligand should be taken into consideration when analyzing experimental results. Metal ions present in living cells and the formation of coordination compounds influence the character of noncovalent interactions between bioligands. Indeed, the centers of such interactions are at the same time the sites of metal–ligand bonds [5].

Metal ions influence structure and activity of biomolecules significantly affecting the charge distribution pattern and intermolecular interactions. The action of metal ions is different for various ions and depends primarily on their binding sites and molecular conformation. Alkali metal ions as Mg(II) or Ca(II) bind preferentially to the phosphate groups and stabilize the DNA double helix. Transition metal ions like Cu(II) or Zn(II) bind to nucleic acid basis leading to DNA denaturation by destroying the hydrogen bond structure. Copper(II) ion is an essential trace element being the internal component of many enzymes. This ion is required for growth and development of many organisms [20]. However, a binding of Cu(II) to some bioactive molecules can result in enhancing of their toxicity [21]. It is generally assumed, that metal ions are preferably coordinated by oxygen atoms of phosphate groups. However, the enhanced stability of Cu(II) complexes with adenosine nucleoside and NMR spectra suggest that nitrogen N7 of purine residue can be directly involved in the coordination [22].

Knowledge of the binding sites, complex geometry and its electronic structure are essential for any microscopic model of ATP activity. It is generally known that nucleosides are polydentate ligands with various potential binding sites, which are related to: negatively charged oxygen atoms of the phosphate group, nitrogen atoms N1 and N7 of the adenine, and hydroxyl groups of the ribose sugar [5].

When polyamine molecules appear in a solution then three additional Cu(II) coordination sites appear at NH_2 and NH groups of 3,3-tri and spermidine (see Scheme 1). These polyamines are of tridentate type. We have recently studied interactions and Cu(II) coordination properties in ternary systems with linear bidentate polyamines tn = 1,3-diaminopropane and putrescine [23]. Yet, our understanding of the modes of action of Cu(II) ions at the molecular level is still not satisfactory. This is due to the complexity of the studied systems with various competing intermolecular interactions. There exist different coordination sites with different binding properties and a competition between aggregation of large biomolecules and Cu(II) coordination. Various type complexes of different stability are formed in various conditions depending on pH-value, relative concentration of components, metal charge compensation mechanism [24] and temperature. Most of studies have been performed in water solutions, but usually the water molecules are not closely considered as an active coordination species.

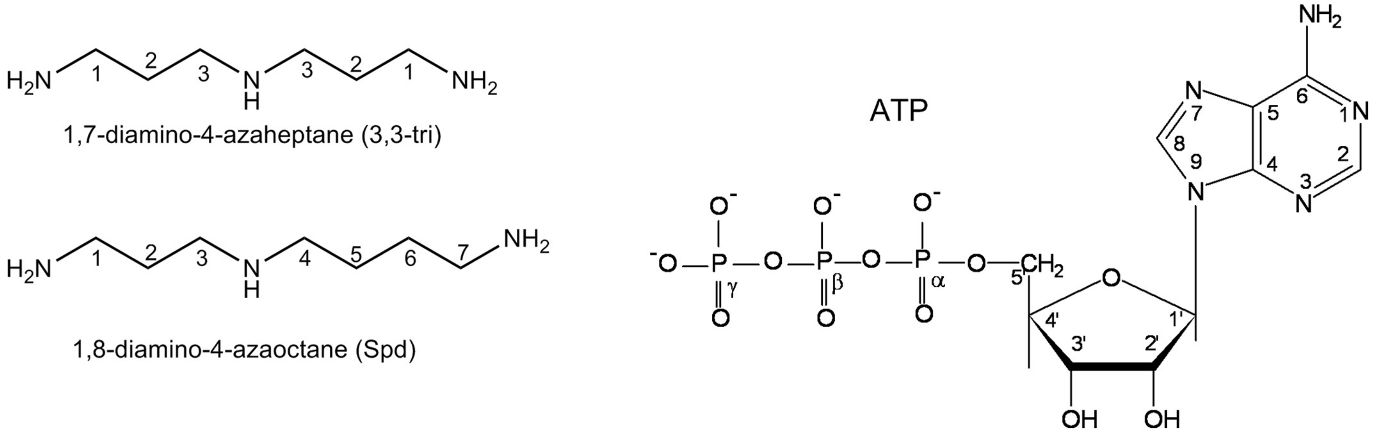
Except various experimental techniques used in investigations of interactions of Cu(II) with biomolecules the theoretical calculations of molecular geometry and possible binding sites were performed by Molecular Orbital (MO) theory for nucleic acid bases [25], by DFT calculations for compounds of aminoacids and nucleobases [26], and by Ligand Field Molecular Mechanics method for Cu(II) amine complexes [27]. However, a consistent picture of Cu(II) coordination obtained from various methods has been not achieved.

In this paper we describe interactions between ATP and polyamines (PA) spermidine (Spd = 1,8-diamino-4-azaoctane) and between shorter analogue of Spd, i.e. 1,7-diamino-4-azaheptane = 3,3-tri in water solutions. Their molecular formulae are schematically presented in Scheme 1. We have analyzed potentiometry and NMR results for identification of intermolecular noncovalent interactions, which are responsible for stacking of ATP and PA biomolecules. When Cu(II) is added, a competition between intermolecular interaction and coordination interaction appears and various Cu(II) complexes are formed depending on component concentrations and pH value of the studied solution. Because of controversial results of the published data we present electron paramagnetic resonance (EPR) and optical spectra of Cu(II) first for binary systems Cu-polyamine and Cu-ATP (i.e. separately for Cu-phosphate, Cu-adenosine and Cu-ribose). The analysis of the spectra indicates possible Cu binding sites in the ternary Cu/ATP/PA system. It should be stressed that EPR measurements were performed after rapid freezing at liquid nitrogen temperature (77 K) i.e. in a solid glassy state, where anisotropic EPR spectra are well resolved. It is generally assumed that the rapid freezing allows an observation of an instantaneous state of the solution. However, we have observed a significant discrepancy between a picture observed by potentiometry at liquid state and a simpler picture observed by EPR spectroscopy at glassy state. This problem is still very weakly recognized.

2. Experimental

2.1. Materials

Adenosine-5′-triphosphate sodium salt (ATP) (purity 99%), 1,7-diamino-4-azaheptane (3,3-tri) – $C_6H_{17}N_3$ (purity 98%) and 1,8-diamino-4-azaoctane - spermidine (Spd) – $C_7H_{19}N_3$ (purity 97%) was purchased from Sigma-Aldrich. Polyamine nitrates were prepared by dissolving appropriate amounts of free amine in methanol followed by adding equimolar amounts of HNO_3. The obtained white precipitates were recrystallized, washed with methanol and dried in air. The ligands were subjected to elemental analysis, results of which (3,3-tri 22.51% C, 25.94% N, 6.10% H and Spd 25.32% C, 24.87% N, 6.61% H) were in



Scheme 1. Chemical formulae of the bioligands studied.