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Influence of copper(II) ions on the noncovalent interactions between cytidine-5′-diphosphate or cytidine-5′-triphosphate and biogenic amines putrescine or spermidine

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ABSTRACT

Potentiometric and NMR spectroscopic studies of the nucleotide (NucP)/polyamine (PA) system (where NucP = *CDP*, *CTP*, PA = putrescine or spermidine) revealed the formation of molecular complexes (NucP)(H_{x+y})(PA) (where H_{x+y} = number of protons; x - from NucP and y - from PA). Their thermodynamic parameters were determined and the modes of their interactions were proposed. The main reaction centers were found to be the protonated amine groups of polyamine (positive centers) and phosphate groups of nucleotide (negative centers). The pH ranges in which the complex occurs correspond to those of amine protonation and $-PO_3^{x-}$ group deprotonation, which unambiguously confirms the dipole-dipole type of interaction. In the pH range of total deprotonation of $-NH_x^+$ groups from the polyamine, the molecular complexes disappear. The equilibrium and spectroscopic studies of the ternary systems Cu(II)/NucP/PA evidenced the formation of Cu(NucP)H_{x+y}(PA) type coordination compounds and Cu(NucP)···(PA)(H_x) type molecular complexes with polyamine in the outer coordination sphere. The main sites of metal ion bonding in the latter species are the phosphate groups of the nucleotide, while in the coordination compounds – besides the phosphate groups – also the donor nitrogen atoms from the polyamines. In this paper we have also quantitatively calculated the effect of metal ions on the formation of the molecular complexes.

1. Introduction

Polyamines (PA), i.e. 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 high concentrations in the cells of all living organisms [1–4] and in the form of polycations react with the negative fragments of DNA, RNA or proteins, which determines the cell growth and proliferation [5,6]. The presence of polyamines leads to changes in the structure of acids, which affects the process of gene expression and transfer of genetic information. The concentration of amines in an organism depends on the type of tissue and age of cells. Relatively high concentration of polyamines (~2 mM of spermidine or 4 mM spermine) was found in pancreas [7]. A particularly important observation was that the level of PAs increased in cancer cells, which permits monitoring of the disease progress and indicates the direction of the search for chemotherapeutic substances [8]. Polyamines have also been indicated as involved in the Alzheimer's disease or infections [9–11]. Unfortunately, their role in the processes taking place in a living organism has not been fully explained as yet [6]. One of the main problems that has to be solved is whether the charge of the polycation or its structure decides about the character of noncovalent interactions in living organisms also in the reactions with metal ions present in the cells. Of course, 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 [12]. The inhibition of polyamine syntheses in cells results in the retardation of proliferation [5,6]. According to the Manning's polyelectrolyte theory, the mode of interactions depends mainly on the charge of reagents [13,14]. However, this approach does not explain the specificity of certain reactions. Polyamines cannot be treated as a point charge, as can be assumed in the description of the reaction of biomolecules with metal ions, e.g. with magnesium [14]. The structure and spatial matching of polyamine and other bioligand should be taken into account. This study is devoted to the analysis of interactions in the model binary systems CDP/Put, CDP/Spd, CTP/Put and CTP/Spd, as well as in the above systems including Cu(II) ions.

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2. Material and methods

2.1. Chemicals

Cytidine-5′-triphosphate disodium salt (CTP) (98% pure), cytidine-5′-diphosphate disodium salt (CDP) (98% pure), putrescine – 1,4-diaminobutane (*Put*) – $C_4H_{12}N_2$ (99% pure) and 1,8-diamino-4-azaoctane - spermidine (*Spd*) – $C_7H_{19}N_3$ (97% pure) were purchased from Sigma-Aldrich. PA nitrates were obtained by dissolving appropriate amounts of free amine in methanol and adding equimolar amounts of HNO_3 in relation to a number of amino groups. The white precipitate was recrystallized, washed with methanol and dried in air. The ligands in the form of nitrates were subjected to elemental analysis on a Perkin-Elmer Elemental Analyzer CHN 2400. Results of the analysis (Put: 22.24% C, 25.97% N, 6.33% H; Spd: 25.32% C, 24.87% N, 6.61% H) were in a good agreement with theoretically calculated values (± 0.3% apparatus error). Copper(II) nitrate trihydrate $Cu(NO_3)_2{\cdot}3H_2O$ (p.p.a.) was purchased from POCh (Poland) and purified by recrystallization from water. The concentration of Cu(II) ions was determined on a Varian inductively coupled plasma - mass spectrometer (ICP-MS). The CO_2-free NaOH solution, used as a titrant, was prepared from Sørensen solution and its concentration was determined by the ICP-MS method. D_2O, NaOD and DCl were purchased from the Institute for Nuclear Research (Swierk, Poland).

2.2. Potentiometric measurements

Potentiometric measurements were performed using Methrom 702 SM Titrino with an autoburette. A glass Methrom 6.0233.100 electrode was calibrated in terms of hydrogen ion concentration [15] with initial calibration using a phthalate buffer (pH = 4.002) and borax buffer (pH = 9.225). In the metal-free systems, the concentration of ligands was $1{\cdot}10^{-2}$ M, while in the ternary systems with copper(II) ions it was $1 \cdot 10^{-3}$ M. In the metal-free systems the ratio of ligand concentrations NucP:PA (where NucP = *CDP* or *CTP* and PA = *Put* or *Spd*) was 1:1, while in the metal-containing systems the ratio of concentrations Cu(II):(NucP):PA was 1:1.8:1.8 or 1:2.3:2.3. The potentiometric titration was carried out at the constant ionic strength μ = 0.1 M (KNO_3) at 20 ± 1 °C in an inert gas atmosphere (helium), using a solution of NaOH (~0.13 M) as a titrant. The addition of subsequent portions of the NaOH solution did not change the ionic strength as the measurements started from fully protonated polyamine, so that the $-NH_x^+$ cations were replaced by an equivalent number of Na^+ cations. The initial volume of the sample was 30 mL. The model choice and determination of stability constants (logβ) of complexes formed in the systems studied were made with the aid of the program HYPERQUAD [16]. For each system, 6 titrations were made (100–250 points) in the pH range 2–10.5. The analysis started from the simplest hypothesis and then the model was extended by subsequent species and the results were verified by the procedures described in Ref. [17]. These procedures were applied to the binary (metal-free) systems as well as to the Cu(II) ion-containing ternary systems. The hydrolysis constants of Cu(II) were taken from literature [18].

2.3. Spectral measurements

The mode of interactions was established on the basis of spectroscopic measurements carried out in the pH range in which particular species were dominant, on the basis of the equilibrium studies. The distributions of particular species were obtained using the program HALTAFALL [19].

2.3.1. NMR spectroscopy

Samples for ^{13}C NMR and ^{31}P NMR studies were prepared by dissolving appropriate amounts of the ligands in D_2O. The pD values were set with the use of DNO_3 and NaOD solutions. Readings from a pH meter N517 (Meratronik, Poland) were corrected according to the formula $pD = pH_{readings} + 0.40$ [20]. The ligand concentration in the samples was 0.1 M and the concentration ratio of NucP:PA = 1:1 in the metal-free systems, The ^{13}C NMR spectra were recorded on an NMR Gemini 300VT Varian spectrometer using dioxane as an internal standard. The ^{13}C NMR signal positions were recalculated on the TMS scale. The ^{31}P NMR spectra were recorded on an NMR spectrometer Varian Unity 300 with H_3PO_4 as an internal standard.

2.3.2. Vis spectroscopy

The Vis spectra were recorded on a UV/Vis Thermo Fisher Scientific Evolution 300 spectrophotometer. The samples were prepared in H_2O and the ligand concentrations were the same as those used in the potentiometric studies at the ratio Cu:NucP:PA = 1:1.8:1.8. A Plastibrand PMMA cell with 1 cm path length was employed in the measurements.

2.4. Quantum chemical calculations

The calculations of charge at the potential interaction centers were performed with GAUSSIAN-09 [21]. The calculations were made for the ground electronic state using the Density Functional Theory (DFT) at three-parameter hybrid functional B3LYP and standard basic set 6–31 + G(d,p).

3. Results and discussion

The formulae of the studied nucleotides (NucP), i.e. *CDP* or *CTP*, and polyamines (PA), i.e. *Put* or *Spd*, are shown in Scheme 1.

3.1. Interactions in the metal-free NucP/PA systems

Results of equilibrium and spectroscopic (NMR) studies have shown that the molecular complexes (adducts) of the general formula (NucP)$\cdots$(H_xPA), where x = the number of H^+, are formed in the studied binary systems as a consequence of noncovalent interactions between cytidine-5′-diphosphate (*CDP*) or cytidine-5′-triphosphate (*CTP*) and putrescine (*Put*) or spermidine (*Spd*). The formation of these species is accompanied by the liberation of protons and a shift in the acid-base equilibrium of the ligands, which permits the determination of the model and the thermodynamic stability. The overall stability constants of the complexes (logβ) and stoichiometric composition of the studied species were calculated on the basis of potentiometric measurements subjected to computer analysis with the use of the least square methods (HYPERQUAD). Due to the differences in the number of protons in particular species (e.g. the value of β in (NucP)H_2(PA) and (NucP)H_3(PA) complexes has different dimensions) the analysis of thermodynamic stability was made not directly with logβ values (as has been sometimes erroneously made in such a discussion). We used the stability constants of formation, calculated for the general reaction H_x(CDP) + H_y(PA) ⇆ (CDP)H_{x+y}(PA): $\log K_e = \log\beta_{(CDP)Hx+y(PA)} - \log\beta_{Hx(CDP)} - \log\beta_{Hy(PA)}$, where the subsequent values of β are the overall stability constants of complexes, overall protonation constants of *CDP* and polyamine, respectively (Supplementary Table S1). The values of K_e depend to the energy of ligand binding in the adduct. In the systems studied the formation of adducts containing 2–4 protons was found (Table 1).

The considered equilibria of adduct formation have been selected by analyzing distribution curves presenting ranges of the occurrence of free bioligands in relation to pH and comparing these ranges with those of the formation of molecular complexes. In the case of ternary complexes, the ranges of the occurrence of binary complexes were also analyzed. For example, it is evident that the complex (CTP)H_4(Spd) which occurs from pH of about 3 is a product of the reaction between very basic H_3(Spd), which also occurs from pH of about 3, and monoprotonated species HCTP with the same distribution as the adduct. Both species disappear at pH of about 8, Fig. S1 (for equilibrium reaction see in Table 1).