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Kinetic studies of the reactions between dichlorido(1,2-diaminoethane)zinc(II) and biologically relevant nucleophiles in the presence of chloride

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ABSTRACT

The mole-ratio method was used for determining metal–ligand stoichiometry for the reaction between $[ZnCl_2(en)]$ (where en = 1,2-diaminoethane or ethylenediamine) and chloride ion at pH 7.2. The results have shown step-wise formation of 1:1 and 1:2 complexes and indicate additional coordination of chloride ions in the first coordination sphere. The kinetics of ligand substitution reactions of the zinc(II) complex and biologically relevant nucleophiles such as inosine-5′-monophosphate (5′-IMP), guanosine-5′-monophosphate (5′-GMP), L-methionine (L-Met), glutathione (GSH) and DL-aspartic acid (DL-Asp) were investigated at pH 7.2 as a function of nucleophile concentration in the presence of 0.010 M NaCl. The reactions were followed under pseudo first-order conditions by UV-Vis spectrophotometry. The substitution reactions include two steps of consecutive displacement of chlorido ligands and changes in coordination geometry. In the presence of an excess of chloride, the octahedral complex anion $[ZnCl_4(en)]^{2-}$ formed. The first step of the substitution reactions could be interpreted as substitution of the axial chlorido ligands in the *cis* position to bidentate ethylenediamine by the biologically relevant nucleophiles, while the second step could be interpreted as substitution of the equatorial chlorido ligand. The order of reactivity of the investigated nucleophiles for the first reaction step is 5′-IMP > GSH > L-Met > DL-Asp > 5′-GMP, while for the second reaction step the order of reactivity is GSH > L-Met > 5′-IMP > DL-Asp > 5′-GMP.

KEYWORDS: zinc(II) complex, biologically relevant nucleophile, excess chloride

1. INTRODUCTION

Transition metal ions play an important role in metalloenzymes; they act mainly as Lewis acids, do not change their oxidation state nor, generally, their protein ligands. Changes in the coordination sphere may occur on the side exposed to solvent.

Zinc has a specific role in bioinorganic processes because of the peculiar properties of the coordination compounds of the zinc(II) ion. Thus, it can easily be four-, five-, or six-coordinate, without a marked preference for six-coordination [1]. The most studied metalloproteins, in which zinc serves a structural role, belong to the zinc-finger family, which is involved in control of nucleic acid replication, transcription and repair [2]. In zinc-finger proteins, zinc is tetrahedrally coordinated to histidines and/or cysteines. Coordination of aspartic acid and glutamic acid residues to the metal has also been found in metalloenzymes [3].

As a catalyst in enzymes, zinc is exposed to the solvent, which for enzymes is almost always water. A coordinated water molecule exchanges rapidly, because ligands in zinc complexes

are kinetically labile. This can be accounted for by zinc's lack of preference for a given coordination number. A six-coordinate complex can experience ligand dissociation, giving rise to a five-coordinate complex with little energy loss and a low energetic barrier [4]. Zinc is a good Lewis acid, especially in complexes with lower coordination numbers; it lowers the p*K*a of coordinated water and is kinetically labile and the interconversion among its four-, five- and six-coordinate states is fast [5]. Theoretical studies have shown that zinc does not have a strong preference for a particular number of water molecules in its first coordination sphere and can accommodate four, five, or six water ligands; the calculated energy differences between isomeric $[Zn(H_2O)_6]^{2+}$, $\{[Zn(H_2O)_5].(H_2O)\}^{2+}$ and $\{[Zn(H_2O)_4].(H_2O)_2\}^{2+}$ complexes are only a few kilocalories per mole [6]. The investigation of the free energies of isomerisation between six- and four-coordinate structures containing Zn^{2+} bound to water and ligands of biological interest has shown that the lowest-energy ground-state coordination number of zinc bound to one acidic or two or more neutral protein ligands is likely to be four. Hydrated zinc with a coordination number of six undergoes a change in its coordination geometry upon binding to the first or second amino acid residue. The observed decrease in the coordination number of zinc upon protein binding reflects primarily the requirements of the metal and ligands, rather than the constraints of the protein matrix on the metal. This is partly due to the greater charge transfer to zinc from more complex ligands compared to that from water [7].

The main goal of this study was to investigate the kinetics and mechanism of ligand-substitution reactions between a model zinc(II) complex such as tetrahedral $[ZnCl_2(en)]$ (where en = 1,2-diaminoethane or ethylenediamine) and biologically relevant nucleophiles, under physiological conditions. The detailed mole-ratio study was used to determine the metal–ligand stoichiometry in the presence of an excess of chloride and kinetic studies at pH 7.2 were performed. It was envisaged that this study could throw more light on understanding the changes in geometrical structures of zinc(II) that relate to structure–reactivity correlation and also to provide more information about biological reactivity preferences of selected biomolecules towards zinc(II). The structures of the complex and the selected nucleophiles are shown in Figure 1.

2. EXPERIMENTAL

2.1 Chemicals

The nucleophiles inosine-5′-monophosphate (sodium salt hydrate, 5′-IMP), guanosine-5′-monophosphate (disodium salt hydrate, 5′-GMP), L-methionine (L-Met), DL-aspartic acid (DL-Asp) and glutathione (GSH) and the buffer 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) were obtained from Sigma-Aldrich, Acros Organics and Fluka. Nucleophile stock solutions were prepared shortly before use by dissolving the chemicals in purified water. All other chemicals were of analytical reagent grade. Highly purified deionised water was used in the preparation of all solutions. For the investigation of the ligand substitution reactions at pH 7.2, a freshly prepared Hepes buffer (0.025 M) was used. NaCl was used to adjust the chloride concentration.

2.2 Synthesis of complex

The complex $[ZnCl_2(en)]$ was synthesised according to the literature method [8]. Chemical analysis was performed on a Carlo Erba 1106 elemental analyser: Anal. calcd for $[ZnCl_2(en)]$:

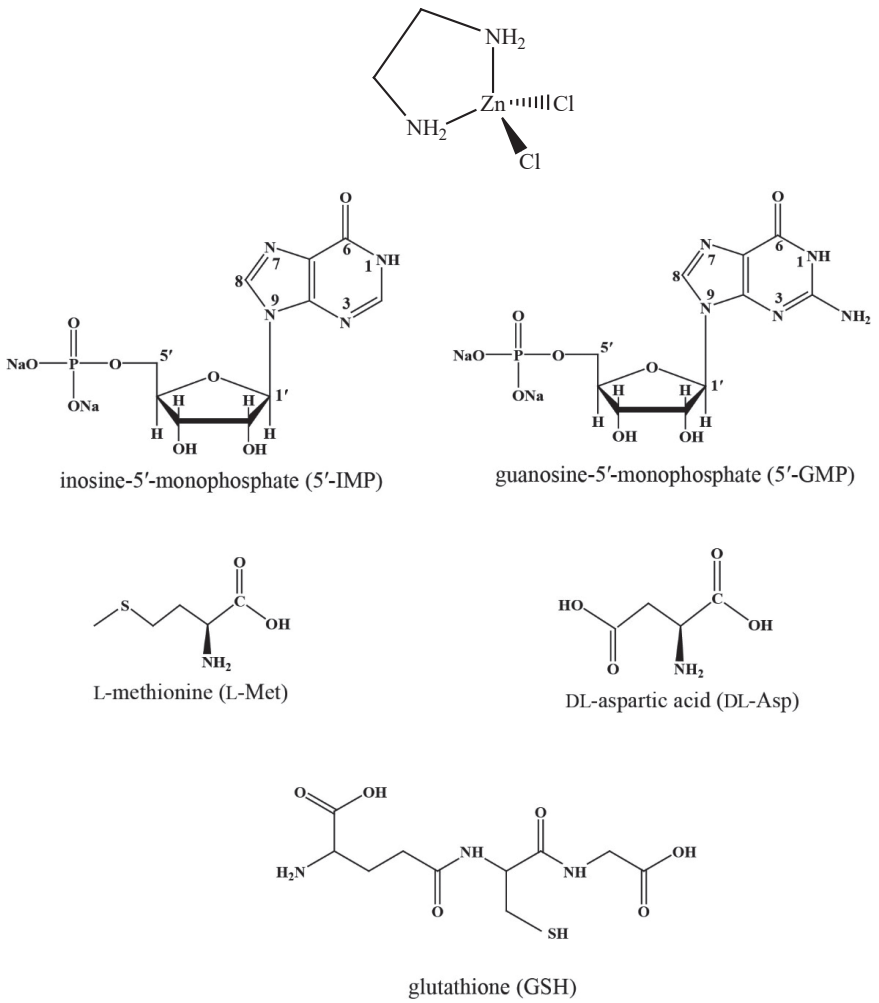


Figure 1 Structures of investigated complex and nucleophiles along with adopted abbreviations.

N, 14.26; C, 12.23; H, 4.11; found: N, 14.29; C, 12.19; H, 4.13%. The geometry of the complex in solution has been investigated and assigned as tetrahedral [9].

2.3 Instrumentation

UV-Vis spectra were recorded on Uvikon XS and Shimadzu UV250 diode-array spectrophotometers in thermostated 1.00 cm quartz Suprasil cells. The temperature was controlled throughout all kinetic experiments to ± 0.1 °C.

2.4 Mole-ratio method

In order to determine the metal–ligand stoichiometry, a series of solutions (10 mL) was prepared in which the concentration of the [$ZnCl_2$(en)] complex was held constant (0.001 M) while the concentration of chloride was varied in different molar ratios ([Cl^-] /[$ZnCl_2$(en)] = 1, 2, 3, 5, 10) [10,11]. Hepes (0.025 M) was used as a buffer at pH 7.2. The absorbance of each solution was measured over the wavelength range 200 to 400 nm (see Figure 2). The chosen absorbance at

234 nm was plotted *versus* the molar ratio of the reactants (see Figure 3). Assuming the complex formed absorbs more than the initial reactants, this plot produces an increasing absorbance up to the combining ratio. At this point, further addition of chloride will produce less increase in absorbance. Thus, a break in the slope of the curve occurs at the mole-ratio corresponding to the combining ratio of the chloride/complex.

2.5 Kinetic measurements

Spectral changes resulting from mixing [$ZnCl_2$(en)] and nucleophile solutions were recorded over the wavelength range 200 to 800 nm to establish a suitable wavelength at which kinetic measurements could be performed [see Figure 4 and the Electronic Supplementary Information (ESI) Tables S1–S5]. The ligand substitution reactions were studied for the nucleophiles 5′-IMP, 5′-GMP, L-Met, GSH and DL-Asp. Reactions were initiated by mixing equal volumes of the complex and ligand thermostated solutions in the UV-Vis spectrophotometric cell and were followed for at least eight half-lives. All kinetic experiments were performed under pseudo first-order conditions with respect to the nucleophile concentration. The observed pseudo first-order rate constants, k_{obsd}, were calculated as the average value from three to four independent kinetic runs and are summarised in Tables S1–S5 in the ESI. All kinetic runs could be fitted to a double exponential function. The reactions were studied at pH 7.2 (0.025 M Hepes buffer) at 295 K in the presence of 0.010 M chloride concentrations. The initial concentration of [$ZnCl_2$(en)] was 0.0001 M.

3. RESULTS AND DISCUSSION

An alternative to the method of continuous variations (Job's method) for determining the stoichiometry of metal–ligand complexes is the mole-ratio method in which the amount of one reactant, usually the molar concentration of metal, is held constant while the amount of the other reactant is varied [10,11]. In order to determine metal–ligand stoichiometry for the reaction between the [$ZnCl_2$(en)] complex and chloride ion at pH 7.2, the absorbance changes over the wavelength range 200 to 400 nm for different molar ratios [Cl^-]/[$ZnCl_2$(en)] were recorded (see Figure 2). The differences between the spectra of the [$ZnCl_2$(en)] complex and those of solutions with various molar ratios [Cl^-]/[$ZnCl_2$(en)] can only be seen in the intensity of the absorbance and the maximum has been shifted from 213 to 227 nm due to coordination of the chloride. The existence of mixed Cl–H_2O complexes was not observed.

The absorbance was monitored at a wavelength where the metal–ligand complex absorbs. At the selected wavelength (234 nm) we observed two equivalence points, corresponding to step-wise formation of 1:1 and 1:2 complexes (see Figure 3).

The results obtained were analysed according to improved techniques for applying the mole-ratio method to the identification of weak complexes in solution (see the ESI) [11]. All three complex species, [$ZnCl_2$(en)], [$ZnCl_3$(en)]$^-$ and [$ZnCl_4$(en)]$^{2-}$, absorb at the selected wavelength. The coordination number of Zn^{2+} is changed from four up to six and accordingly the geometries of the complexes formed undergo change (Scheme 1) [1,7,9].

In the presence of 0.010 M NaCl we assumed that the octahedral complex anion [$ZnCl_4$(en)]$^{2-}$ is formed rapidly and all substitution processes of this complex species should be considered.

All kinetic experiments in this study were performed at physiological pH and 295 K, in the presence of chloride. An example of the UV-Vis spectra and time profile are shown in Figure 4. The substitution reactions of the studied complex and bioligands proceed in two consecutive

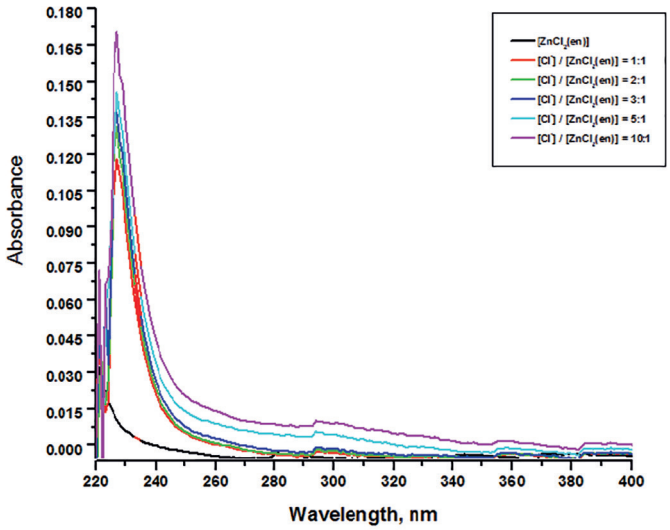


Figure 2 Absorbance changes for reactions between [$ZnCl_2$(en)] complexes (0.001 M) and chloride in different molar ratios at pH 7.2 (0.025 M Hepes buffer) and 295 K.

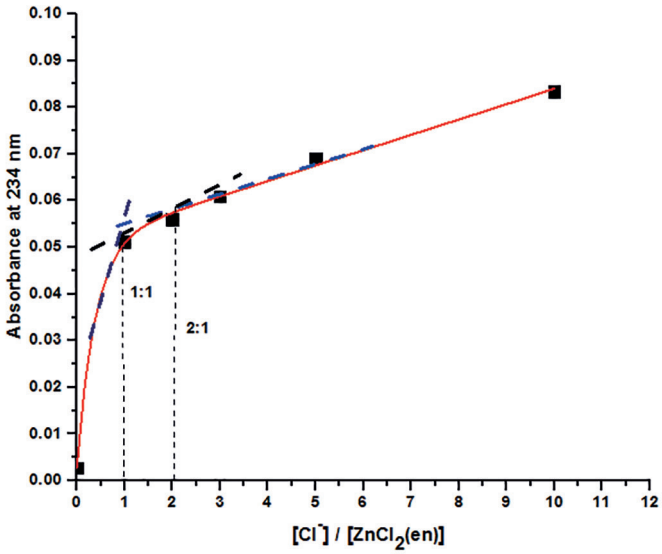
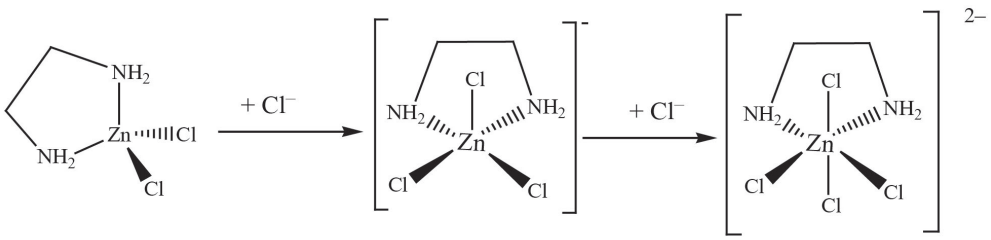


Figure 3 Stoichiometry of chloride–[$ZnCl_2$(en)] complexes using the mole-ratio method.



Scheme 1 Step-wise formation of 1:1 and 1:2 complexes in the presence of excess chloride.

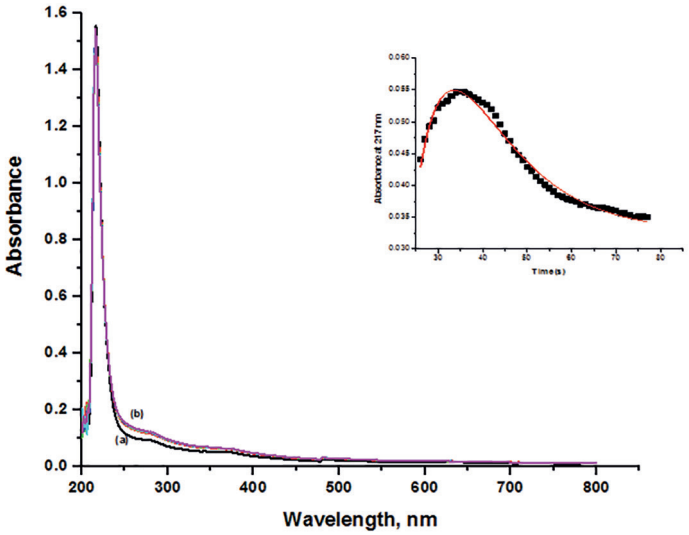


Figure 4 Rapid-scan spectra recorded for reaction of [$ZnCl_2$(en)] complex (0.0001 M) with L-methionine (0.003 M) at pH 7.2 (0.025 M Hepes buffer) in the presence of 0.010 M NaCl at 295 K (a) before the reaction, (b) after mixing of the reactants. Insert: time profile obtained for reaction at 217 nm.

reactions steps that both depend on the nucleophile concentration. All kinetic traces gave an excellent fit to a double exponential function, typical for a two-step reaction. The so-obtained pseudo first-order rate constants, k_{obsd1} and k_{obsd2}, calculated from the kinetic traces, were plotted against the concentration of the entering nucleophiles. A linear dependence on the nucleophile concentration was observed for all the studied reactions (see Figure 5) and only in the case of the DNA constituents (5′-IMP and 5′-GMP) was a small intercept observed.

The observed pseudo first-order rate constants, k_{obsd1} and k_{obsd2}, depend on the entering nucleophile (Nu) concentration as given in Eqns (1) and (2).

$$k_{obsd1} = k_1[Nu] + k_{-1} \quad (1)$$

$$k_{obsd2} = k_2[Nu] + k_{-2} \quad (2)$$

The observed linear fits pass through the origin for some reactions in the present study, indicating that possible parallel or reverse reactions are insignificant or absent, *i.e.* k_{-1} and k_{-2} are negligible and Eqns (1) and (2) simplify to $k_{obsd1} = k_1[Nu]$ and $k_{obsd2} = k_2[Nu]$. Thus, in the present systems, direct nucleophilic substitution is the major observed reaction pathway under the selected conditions. The observed intercepts for 5′-IMP and 5′-GMP are ascribed to the reverse reaction with excess chloride present in solution (Figure 5). The derived rate constants are summarised in Table 1.

The first step of the substitution reactions could be interpreted as substitution of the axial chlorido ligands in the position *cis* towards bidentate coordinated ethylenediamine by the nucleophiles, whereas the second step could be interpreted as substitution of the equatorial chlorido ligand. Due to the large negative inductive effects of amino groups in ethylenediamine, the basicity of N-donor atoms increases and the interactions between Zn^{2+} and $–NH_2$ groups are stronger. According to this, both chlorido ligands in axial positions are kinetically labile and

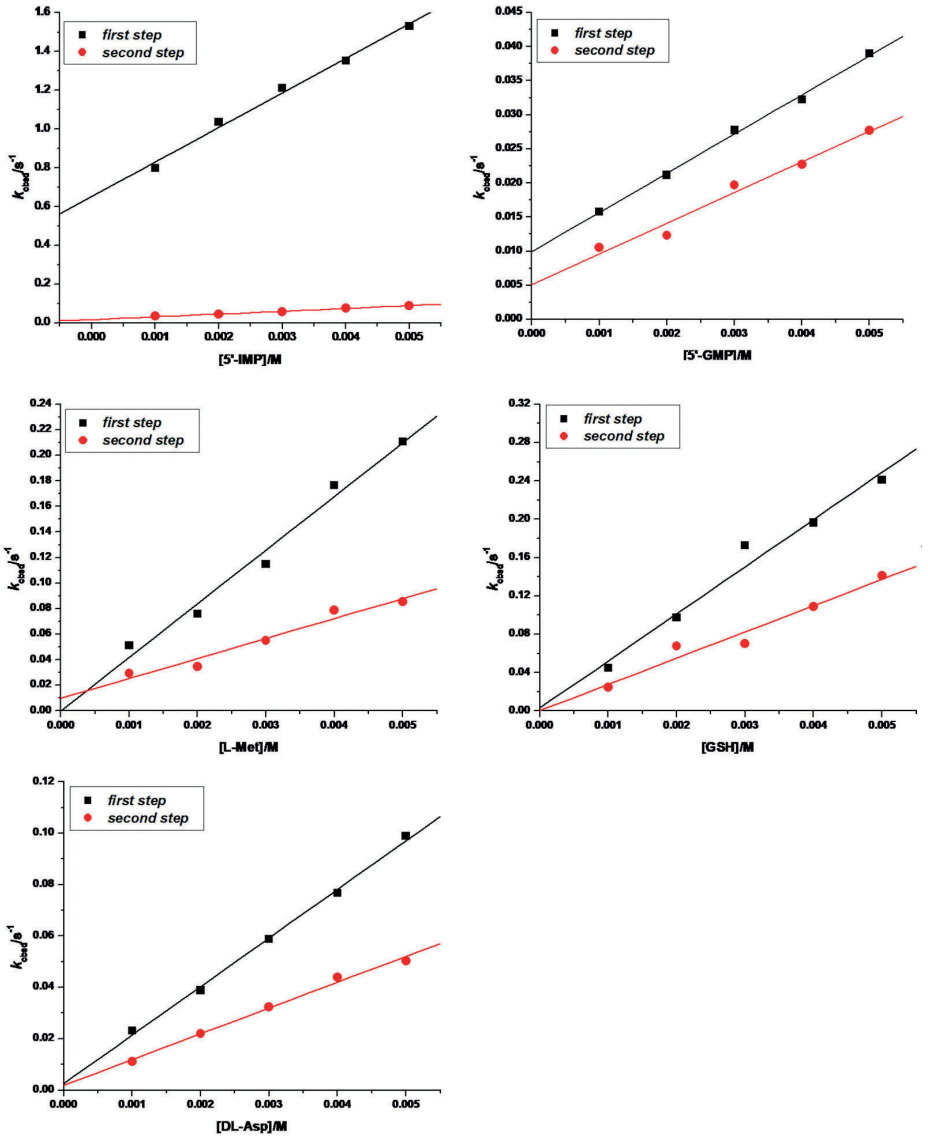
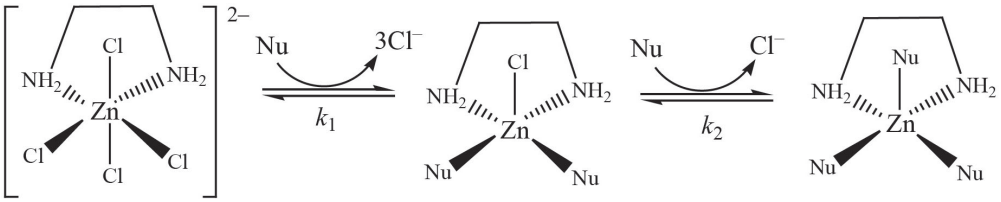


Figure 5 Pseudo first-order rate constants plotted as a function of nucleophile concentration for first and second substitution reactions of [$ZnCl_2$(en)] complex by 5′-IMP, 5′-GMP, L-Met, GSH and DL-Asp at pH 7.2 (0.025 M Hepes buffer) in the presence of 0.010 M NaCl at 295 K.

Table 1 Second order rate constants for the first and second substitution reactions between [$ZnCl_2$(en)] complex and 5′-IMP, GSH, L-Met, DL-Asp and 5′-GMP at pH 7.2 (0.025 M Hepes buffer) in the presence of 0.010 M NaCl at 295 K

[$ZnCl_2$(en)] 0.0001 M	k_1^{295} (M^{-1} s^{-1})	10^2 k_{-1}^{295} [Cl^-] (M^{-1} s^{-1})	k_2^{295} (M^{-1} s^{-1})	10^3 k_{-2}^{295} [Cl^-] (M^{-1} s^{-1})
5′-IMP	178 ± 9	65 ± 3	14 ± 1	17 ± 4
GSH	49 ± 5	–	27 ± 3	–
L-Met	42 ±3	–	16 ± 2	10 ± 2
DL-Asp	19 ± 1	–	10 ± 1	–
5′-GMP	5.7 ± 0.2	9.9 ± 0.1	4.5 ± 0.2	5.1 ± 0.9



Nu = 5′-IMP, 5′-GMP, GSH, L-Met, DL-Asp

Scheme 2 Proposed pathways for reactions between the formed $[ZnCl_4(en)]^{2-}$ complex and biologically relevant nucleophiles in the presence of 0.010 M NaCl.

equal for parallel substitution routes. We assume that the first reaction step is accompanied by dissociation of one chlorido ligand in the equatorial position and a five-coordinate complex has been obtained. The dissociation of ligands in the six-coordinate complex of zinc(II) and formation of a five-coordinate complex occurs with little energy loss. On the other hand, four-coordinate complexes can add a fifth ligand with a low energetic barrier [1,12,13].
The second reaction step could be interpreted as substitution of the last chlorido ligand. The proposed reaction pathways for the reactions between the formed $[ZnCl_4(en)]^{2-}$ complex and biologically relevant nucleophiles in the presence of 0.010 M NaCl are presented in Scheme 2.
The order of reactivity of the investigated nucleophiles for the first reaction step is 5′-IMP > GSH > L-Met > DL-Asp > 5′-GMP, while for the second reaction step the order of reactivity is GSH > L-Met > 5′-IMP > DL-Asp > 5′-GMP.
Zinc(II) is a borderline hardness Lewis acid and displays high affinity for both nitrogen and oxygen donor atoms as well as for sulfur, depending on the coordination number [14]. It is therefore found to be bound to histidines, glutamates or aspartates and cysteines [1]. The square-pyramidal structure of Zn^{2+} in biological systems prefers *O*-carboxylate, carbonyl and *N*-imidazole donor bioligands. The versatility of coordination of Zn^{2+} and DNA has been found in zinc-finger proteins and as part of nucleic acid polymerases and hydrolases [15]. With the variable coordination geometries (tetrahedral, five-coordinate, octahedral) that zinc(II) is able to adopt, its balance in donor site preference (N, O) may account for the lowest reactivity of 5′-GMP. Steric hindrance also could be a reason for the similar reactivity of 5′-GMP for both reaction steps [15,16].

4. CONCLUSIONS

In this study, we determined the metal–ligand stoichiometry between the $[ZnCl_2(en)]$ complex and chloride ion at pH 7.2. In the presence of an excess of chloride, the octahedral $[ZnCl_4(en)]^{2-}$ is formed in solution at pH 7.2. The substitution reactions of this complex and biologically relevant nucleophiles proceed in two consecutive reactions steps that both depend on the nucleophile concentration. The first reaction step is accompanied by dissociation of one chlorido ligand in the equatorial position and a five-coordinate complex is obtained. The second reaction step could be interpreted as substitution of the remaining chlorido ligand. The order of reactivity of the investigated nucleophiles for the

first reaction step is 5′-IMP > GSH > L-Met > DL-Asp > 5′-GMP, while for the second reaction step the order of reactivity is GSH > L-Met > 5′-IMP > DL-Asp > 5′-GMP.

5. ACKNOWLEDGEMENTS

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6. ELECTRONIC SUPPLEMENTARY INFORMATION

The ESI (details of the mole-ratio method and Tables S1–S5) is available through http://ingentaconnect.com/content/stl/prk/2018/00000043/00000001

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7. REFERENCES

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