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Screening of metal complex–amino acid side chain interactions by potentiometric titration

Michael Kruppa, Daniel Frank, Helga Leffler-Schuster, Burkhard König *

Institut für Organische Chemie, Universität Regensburg, D-93040 Regensburg, Germany

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Abstract

Reversible coordination of amino acid side chains to metal complexes is widely used in protein purification (IMAC technique), but available data on affinity and selectivity of such binding processes are limited. We use potentiometric titration of a series of metal complexes with vacant coordination sites in the presence of molecules resembling amino acid side chain functionalities to screen for new affinities. The investigation confirms documented affinities of imidazole to nickel(II) and copper(II) IDA and NTA complexes, and discovers a hitherto unknown binding of zinc(II)- and cadmium(II) cyclen complexes to imidazole.

Keywords: Potentiometric titration; Coordinative binding; IDA; NTA; Cyclen; Binding constant

1. Introduction

In 1975 Porath et al. published a new type of chromatography which was first called, "metal chelate chromatography", but later termed, "immobilized metal (ion) affinity chromatography" (IMAC) [1]. The technique uses the different affinity of proteins to metal complexes immobilized on a chromatographic support [2].

To find suitable metal complexes for this purification method it is necessary to know how strong metal ions are bound to a chelate (problem of metal leaching) [3]. This information can be received using potentiometric titration of the desired metal and chelate [4]. IDA and NTA complexes are well investigated in this respect [5,6], but next to the binding between metal and immobilized chelate, the interactions of substrate and metal complex are essential for their use in chelating purification methods. Weak binding will not generate a good separation. Very strong interactions will block all binding sites. Potentiometric titration can reveal equilibria between metal complexes and additional ligands [7]. The binding of amino acids towards different metal complexes was investigated [8], but in most of the data the α-amine and carboxylate amino acid functional group are used to chelate the metal complex. Working with peptides or proteins, these binding processes are not relevant. C-terminal carboxylate and N-terminal amine of a protein will not chelate a metal complex. In typical peptide conformations, these two coordinative binding sites are too far away from each other to act as a chelating ligand. The additional side chain functionalities are essential for a binding event. The histidine-tag strategy, in which several imidazol side chains and the N-terminal amino group reversibly coordinate to a metal complex, demonstrates this impressively. To identify which side chains of natural amino acids are suitable for metal coordination, potentiometric titration may provide information.

We report here the use of potentiometric titration to screen interactions between several metal complexes (Fig. 1) and functional groups of amino acid side chains. In addition to the well investigated IMAC metal complexes **1**–**4**, we focus our investigation on M(II) cyclen complexes **5**–**8**.

* Corresponding author.
E-mail address: Burkhard.Koenig@chemie.uni-regensburg.de (B. König).

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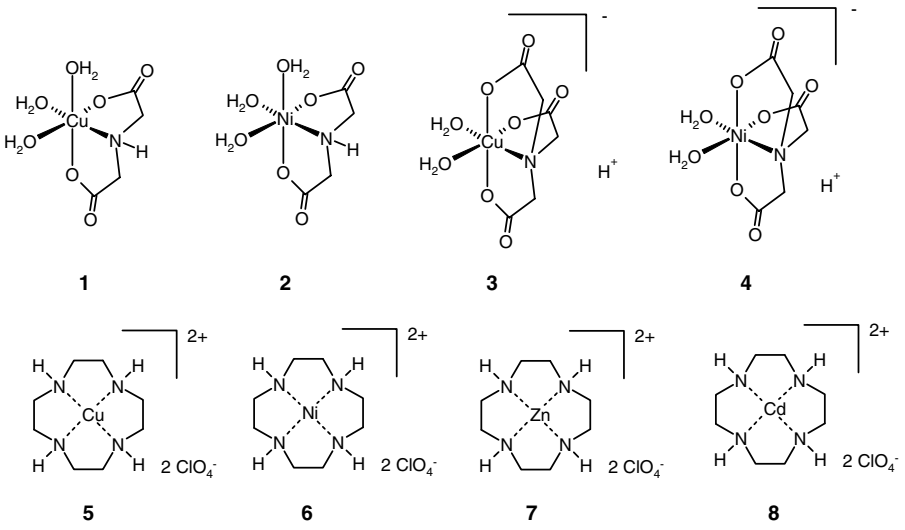

Fig. 1. Structures of metal complexes **1**–**8** investigated in the study.

We represent typical amino acids side chain functional groups by butyl amine (Lsy), acetic acid (Glu, Asp), ethanol (Ser, Thr), ethane thiole (Cys), imidazole (His), *N*-ethylguanidine hydrogenchloride (Arg), phenol (Tyr) and disodium phenylphosphate (phosphorylated Tyr).

The investigation of each metal complex–substrate combination consists of three experiments. In two initial potentiometric titrations, we examine the properties of substrate and metal complex separately. The obtained pK_a values for substrate and metal complex are then used to analyze the titration curve of a 1:1 mixture of substrate and metal complex.

2. Experimental

2.1. General methods and material

TEAOH (Merck), TEAP (Fluka), mono sodium phthalate (Merck) perchloric acid (60%, Merck), iminodiacetic acid (IDA) (Fluka), $Cu_2(CO_3)(OH)_2$ (Alfa Aesar), $NiCO_3 \cdot 2NiOH(OH)_2 \cdot 4H_2O$ (Alfa Aesar), nitirilotriacetic acid (Fluka), cyclen (Schering), copper(II) perchlorate hexahydrate (Alfa Aesar), nickel(II) perchlorate hexahydrate (Alfa Aesar), zinc(II) perchlorate hexahydrate (Alfa Aesar), cadmium(II) perchlorate hexahydrate (Alfa Aesar), ethanol (Merck), acetic acid (100%, Merck), phenol (Merck), imidazole (Fluka), butylamine (Fluka), *N*-ethylguanidine hydrogenchloride (Vocado), di-soidum phenyl phosphate (Fluka) were bought and used without further purification. Melting points (mp) were determined with a Büchi SMP 20 and are uncorrected. IR-spectra were recorded with a Bio-Rad FTS 3000 MX FT-IR as KBr discs. Electro spray mass spectra were recorded on a Finnigan MAT SSQ 7000 ESI-spectrometer. Elemental analyses were measured on a Vario EL III (Elementar Analytical Systems).

2.2. Potentiometric titrations

All titrations were performed under N_2 atmosphere with a computer-controlled pH-meter (pH 3000, WTW) and dosimat (Dosimat 665, Metrohm). For all titrations, 0.1 M perchloric acid and 0.1 M tetraethylammonium hydroxide (TEAOH) in water containing tetraethylammonium perchlorate (TEAP) to maintain an ionic strength of $I = 0.1$ were used. TEAOH solutions were calibrated with mono sodium phthalate. A titration of perchloric acid with TEAOH solution was used for calibration and to determine $\log K_w$. The Irving-factor (A_I) was determined according to $pH_{measured} = pH_{real} + A_I$. All measurements were performed at 25 °C and measurements with detected interactions were repeated to confirm a binding process. Data were analyzed using the program Hyperquad2000 (Version 2.1, P. Gans).

2.3. Synthesis

2.3.1. [Cu(ida)(H_2O)$_2$] (**1**)

IDA (2.40 g, 18.8 mmol) and $Cu_2(CO_3)(OH)_2$, (2.00 g, 9.0 mmol) were dissolved in water (150 mL). The solution was stirred at 60 °C for 2 h. The blue suspension was cooled to room temperature and filtered. Slow evaporation of the solvent gave **1** (8.0 mmol, 1.85 g, 89%) as dark blue crystals. mp > 200 °C. IR (KBr): $\bar{\nu} = 3453\ cm^{-1}$, 3273, 2932, 1574, 1396. Elemental *Anal*. Calc. for $C_4CuH_9NO_6$ (230.66): C, 20.82; H, 3.93; N, 6.07. Found: C, 21.04; H, 3.69; N, 6.02%. X-ray structure analysis of compound **1** in the solid state gave identical results as a previously reported structure analysis [9].

2.3.2. [Ni(ida)(H_2O)$_3$] (**2**)

IDA (1.00 g, 7.5 mmol) and $NiCO_3 \cdot 2NiOH(OH)_2 \cdot 4H_2O$ (0.94 g, 2.5 mmol) were dissolved in water