Abstract

Currently, modified electron transport metaloproteins with presence ion Cu(II) in their active site are most commonly used to study kinetics of electron transfer. One of the most studied model protein is cupredoxin azurin from *P. aeruginosa* with standard redox potential approximately 310–360 nm (depends on experimental conditions and techniques). Ion Cu(II) in azurin is coordinated by the side chain atoms of His^{46}, His^{117}, Cys^{112}, Met^{121} and carbonyl of Gly^{45}. The aim of this work was to prepare macrocyclic ligand, 1-[1*H*-imidazol-2-yl)methyl]-1,4,7-triazacycklononanu (**L**$^{\mathbf{IM}}$), which would be able to form stable complexes with both of copper ions Cu(II) and Cu(I). Such as macrocyclic complexes could be utilized as low molecular models of active sites in proteins. The ligand was prepared using five-step synthesis employing protection of macrocycle, reduction and subsequent bromation of 1*H*-imidazol-2-karbaldehyd, and following alkylation reaction. Deprotection of macrocycle revealed final product of ligand. All reaction intermediates and prepared ligand were characterized by NMR and MS. Potentiometric titrations determined ligand protonation constant (log K_1 = 10,62, log K_2 = 6,65, log K_3 = 4,91), which describe acid-base properties of a ligand in an aqueous solution. Similarly, the coordination properties with selected transition metal ions (Cu^{2+} and Zn^{2+}) were studied. Equilibrium stability constants show the expected high stability of copper complexes, which is a condition for determining the standard redox potential of the pair $[Cu\mathbf{L}^{\mathbf{IM}}]^{2+}/[Cu\mathbf{L}^{\mathbf{IM}}]^{+}$. The behavior of the copper complex in an aqueous solution with different pH according to the speciation of the complex (obtainined by potentiometric titration) was verified spectrophotometrically. Determination of standard redox potential can confirm or exclude the possibility to utilize the succesfully prepared macrocyclic complexes as a model of the azurine active site.

Keywords

electron transfer, azurin, macrocyclic complex, copper