Catalytic Oxidation of Dopamine: Mushroom Tyrosinase vs. Dinuclear Copper(II) Complex

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Abstract
The aim of this scientific study was to compare catecholase activity (representing the ability to oxidize *ortho*-diphenols) of tyrosinase enzyme (isolated from the common mushroom *Agaricus bisporus*) with a completely synthetic dinuclear copper complex toward the neurotransmitter dopamine using standard Michaelis-Menten kinetics model. Both, standard UV–Vis spectrophotometry and amperometry in a batch configuration were presented as complementary methods to study the catecholase activity. Despite the finding of comparable catalytic activity (similar Michaelis–Menten constants), it is necessary to mention that tyrosinase enzyme is not active in methanol solution like investigated dinuclear copper(II) complex.

Key words: Catecholase activity; Dopamine; Tyrosinase; Amperometric detection.

Introduction

Generally, traditional biosensors based on natural enzymes have a short lifetime [1] to be integrated into miniature systems (handheld meters) and could be directly used by patients, like in case of glucometers [2]. Nowadays, the development of biomimetic sensors represents a wide interest of the scientific society in the field of clinical analysis due to promising long lifetime [3]. Numerous scientific papers suggest that a replacement of long-term unstable mushroom (isolated from *Agaricus bisporus*) tyrosinase (TYR) enzyme by copper biomimetic complexes could bring expected improvement [4]. From literature, it is possible to find out that the TYR enzyme forms a tetramer of 119.5 kDa that is composed of two types of subunits, namely H subunits (~43 kDa) and L subunits (~14 kDa). The H subunit contains a binuclear copper-binding site in the oxy-state, in which three histidine residues coordinate each copper ion [5]. In comparison with naturally occurring enzyme, a newly synthesized dinuclear copper complex $[Cu_2L(OH)(H_2O)(EtOH)]^{2+}$ $2ClO_4^-$ with 2,6-bis[(N-methyl piperazine-1-yl)methyl]-4-formyl phenol as ligand (L), which chemical structure is shown in Fig. 1., could have a potential diphenolase (catecholase) activity (see Fig 2) [6]. The aim of this work was to define whether this dinuclear copper(II) complex catalyses an oxidation reaction of dopamine to dopamine-*o*-quinone by air oxygen, as well as the TYR enzyme.

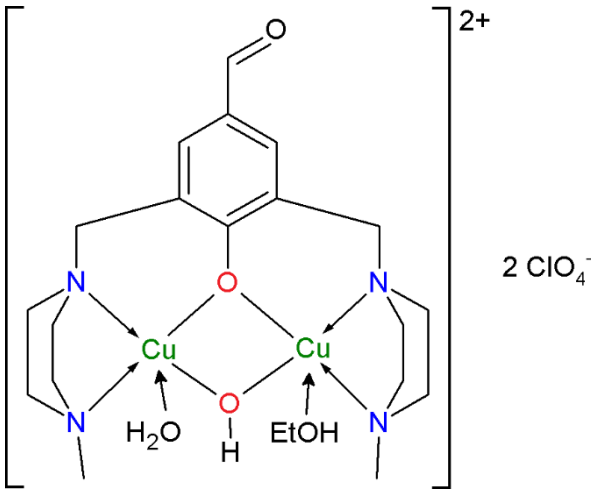

Fig. 1. Chemical structure of investigated dinuclear copper(II) complex.

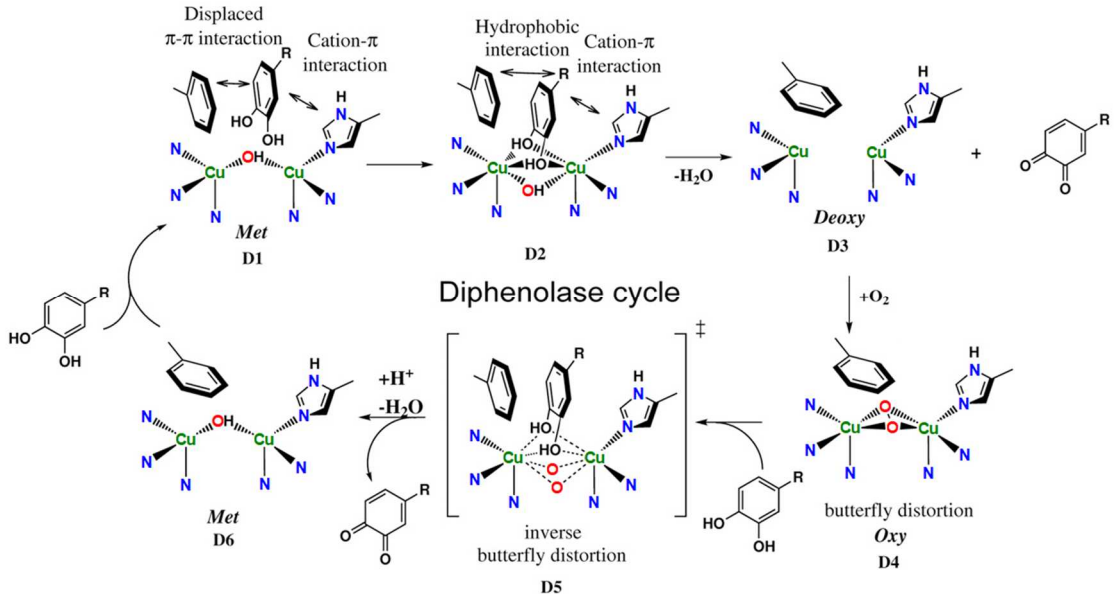

Fig. 2. Diphenolase cycle of mushroom tyrosinase.

Experimental part

Dopamine, *p*-hydroxybenzaldehyde, *N*-methylpiperazine, paraformaldehyde, $Cu(ClO_4)_2 \cdot 6\ H_2O$, triethylamine, 99.99% methanol (MeOH), 99.99 lithium perchlorate were purchased from Sigma-Aldrich (Prague, Czech Republic). All solvents were used without any purification. A deionized water characterized by low resistivity of 18.3 MΩ cm was prepared using Millipore Milli-Q® purification system from Merck KGaA (Darmstadt, Germany).

All measurements in ultraviolet and visible regions of the spectrum were carried out with UV-Vis spectrophotometer UV2450 from Shimadzu (Kyoto, Japan) using 1 cm quartz cuvette from Fisher Scientific (Pardubice, Czech Republic) in the range of wavelengths from 800 to 200 nm at scanning speed of 0.5 nm s^{-1}.

Measurements of kinetics were performed in 3 mL pure MeOH containing 0.1 mol L^{-1} $LiClO_4$ with 150 µmol L^{-1} biomimetic complex, and investigated for concentrations from 100 to 1600 µmol L^{-1} dopamine. At the first, UV–Vis spectrophotometry of the biomimetic complex solution was only measured, and then obtained spectrum was corrected for the blank absorbance signals. For determination of parameters such as Michaelis constant (K_m^{app}) and apparent maximum reaction velocity (V_{max}^{app}), absorbance values at wavelengths 465 nm for dopamine was used.

Each measurement intended for hydrodynamic amperometry was performed in a batch arrangement. Traditional three-electrode cell, consisting GCE (working), saturated calomel electrode (SCE) with 0.1 mol L^{-1} $LiClO_4$ in MeOH as salt bridge (reference) and platinum sheet (auxiliary), were together connected to the AUTOLAB PGSTAT101 potentiostat/galvanostat from Metrohm Česká republika s.r.o. (Prague, Czech Republic), which was operated through NOVA 1.11 software.

Each experiment was performed in a conventional glass cell from International Chemistry Co., LTD. (Matsudo-shi, Japan) containing 500 µmol L^{-1} synthetized dinuclear copper complex in a non-deaerated pure MeOH with 0.1 mol L^{-1} $LiClO_4$ at detection potential of -0.1 V and stirring speed of 400 rpm. Otherwise, all important changes in the working conditions are shown in the figure legends.

Results and Discussion

To determine the wavelength of maximum absorbance of dopamine-quinone which undergoes a nucleophilic reaction (cyclication) to form a leukodopamine-chrome (5,6-dihydroxyindole) [7], UV-Vis spectra of 150 μmol L^{-1} dopamine (black) in the presence of 150 μmol L^{-1} dinuclear copper(II) complex were recorded every 10 min (red lines), as shown in Fig. 3A. The wavelength of 460 nm was set to measure kinetic curves for 100-1600 μmol L^{-1} dopamine (Fig. 3B) which were interpolated by quadratic function. For coefficients of determination (R^2) higher than 0.998, *b*-values of corresponding quadratic equations characterizing the change in absorbance (A) over time (*t*) were used for construction of Michaelis–Menten saturation curve for a catalytic reaction (Fig. 3C). The appropriate Lineweaver-Burk plot is shown in Fig. 3D.

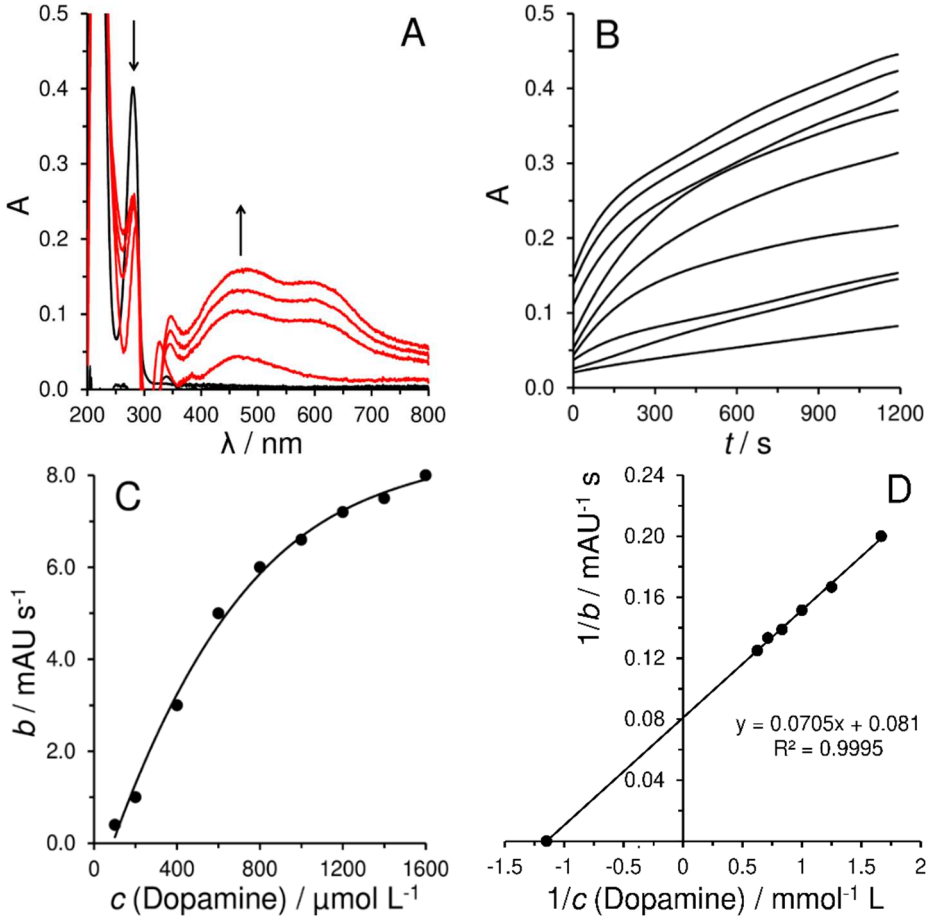

Fig. 3. Spectrophotometric kinetic study of the catecholase activity of $[Cu_2L(OH)(H_2O)(EtOH)]^{2+}$ $2ClO_4^-$ complex towards dopamine oxidation.

If tabulated value of K_m^{app}=0.840 mmol L^{-1} dopamine (BRENDA database) is taken into account for TYR enzyme (*Agaricus bisporus*) in 0.1 mol L^{-1} phosphate buffer (pH 7), the value of K_m^{app}=0.870 mmol L^{-1} dopamine calculated from spectrophotometric measuremnts in pure MeOH containing 0.1 mol L^{-1} $LiClO_4$ indicates the fact that comparable catalytic activity was attained. Moreover, an analogical kinetic study as in the previous case was also carried out amperometrically because the product of dopamine catalytic oxidation (dopamine-quinone) can be electrochemically reduced at 0 (A), -0.1 (B), and -0.2 V. Obtained kinetic curves (D) were linearized (E) to calculate K_m^{app} value, as shown in Fig. 4. The K_m^{app} values of 0.867, 0.763, and 0.776 mmol L^{-1} dopamine was found for 0, -0.1, and -0.2 V respectively. This finding suggests that detection potential does not have any significant effect on

catecholase activity of $[Cu_2L(OH)(H_2O)(EtOH)]^{2+}$ $2ClO_4^-$ complex and both analytical methods used are completely comparable.

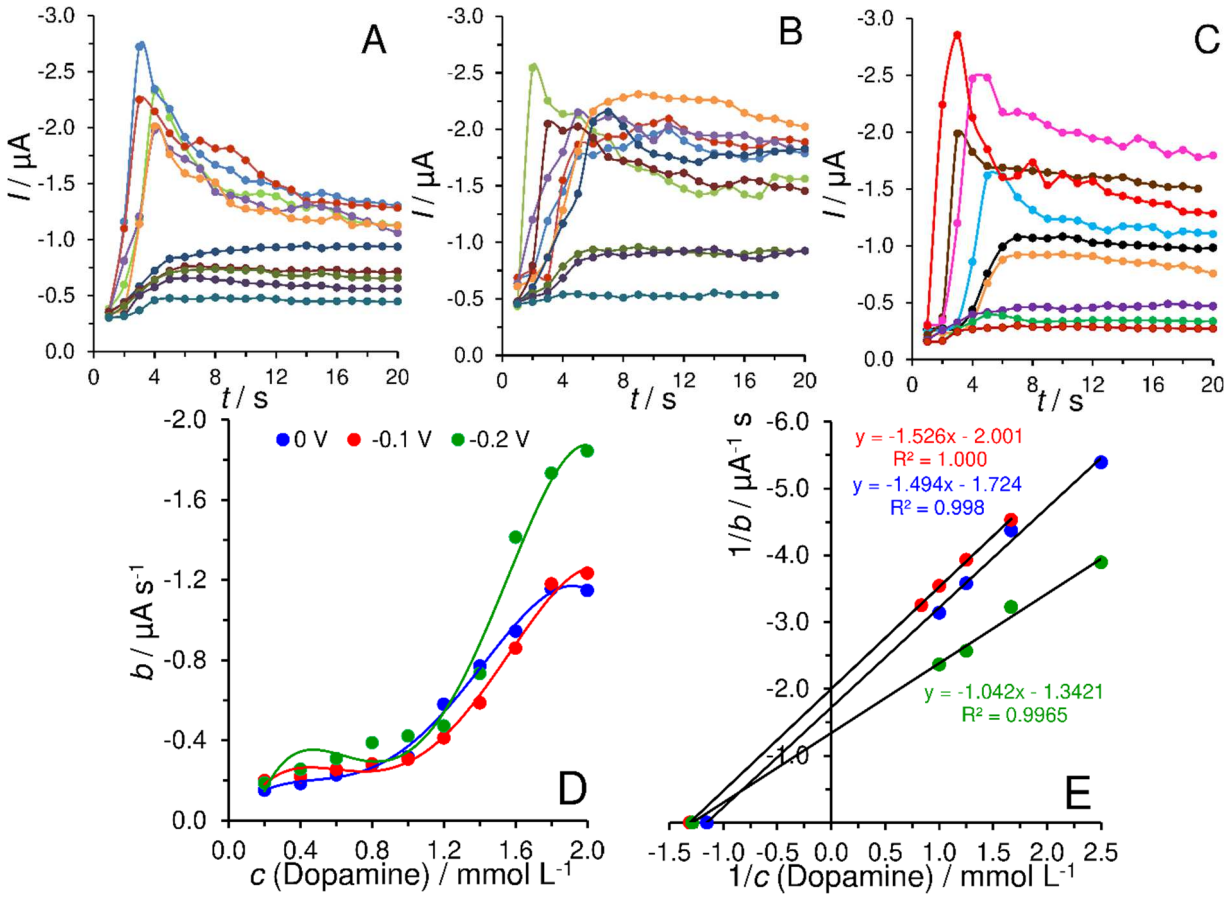

Fig. 4. Amperometric kinetic study of the catecholase activity of $[Cu_2L(OH)(H_2O)(EtOH)]^{2+}$ $2ClO_4^-$ complex towards dopamine oxidation.

Conclusion

The results obtained show that the synthetized dinuclear copper complex provides in pure MeOH similar catalytic (catecholase) activity as natural tyrosinase enzyme in aqueous phosphate buffer of pH 7.0. However, this complex is not stable in phosphate buffer due to probable precipitation of Cu^{2+} cations.

Acknowledgements

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