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Evaluation of Superoxide Dismutate-Like Activity in Some Copper(II) Complexes of Aminoacids

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SUMMARY. The superoxide dismutase-like activity of a series of copper(II) complexes of the aminoacids glycine, alanine, valine, isoleucine, and serine has been investigated using the nitrobluetetrazolium/superoxide reduction assay. The results show that all the investigated complexes possess the capability to dismutate the superoxide anion generated in the xanthine/xanthine oxidase system. The results are compared with those obtained for the native enzyme superoxide dismutase, tested under the same experimental conditions. The lipophilicity of the complexes was tested determining their partition coefficients between physiological solution and n-octanol. The results show an important increment in lipophilicity with increasing complexity of the ligands and also in comparison with that of the free aminoacids.

RESUMEN. "Evaluación de Actividad Superóxido Dismutasa en Algunos Complejos de Cobre(II) con Aminoácidos". Se investigó la actividad superóxido-dismutasa de una serie de complejos de cobre(II) con los aminoácidos glicina, alanina, valina, isoleucina y serina, utilizando el ensayo de reducción del colorante nitroazultetrazolio por superóxido. Los resultados muestran que todos los complejos investigados poseen la capacidad de dismutar al anión superóxido, generado en el sistema xantina/xantina oxidasa. Los resultados se comparan también con los obtenidos para la enzima superóxido-dismutasa nativa, ensayada en las mismas condiciones experimentales. La lipofilicidad de los complejos fue determinada a partir del estudio de sus coeficientes de partición entre solución fisiológica y n-octanol. Se observó un importante incremento en la lipofilicidad al aumentar la complejidad de los ligandos y también en comparación con la de los aminoácidos libres.

It is well established that some copper(II) complexes posses an effective antirheumatic and anti-inflamatory activity [1-3]. On the other hand numerous of these complexes often present potent superoxide dismutase-like activity [1-3].

It is also been suggested that superoxide and related free radicals may contribute significantly to sustaining chronic inflammation by promoting connective-

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KEY WORDS: Superoxide dismutase (SOD); SOD mimics; Copper(II)-aminoacid complexes; Lipophilicity.
PALABRAS CLAVE: Superóxido dismutasa (SOD); Análogos de SOD; Complejos de cobre(II) con aminoácidos; Lipofilicidad.

tissue degradation [4,5]. One possible mode of action of the pharmacologically active copper complexes may be their action as superoxide-dismutase (SOD) mimetics, which destroy extracellular superoxide radicals [5].

As a part of a research project devoted to the synthesis and characterization of copper-complexes with pharmacological activity, we have now prepared a series of complexes of this metal with some aminoacids in order to investigate their general physicochemical and biological properties.

In this paper we report the results of our investigation of the SOD-like activity of these complexes, as well as their lipophilicity.

EXPERIMENTAL

Synthesis of the complexes

Copper(II) complexes of the following aminoacids were prepared: glycine (Gly), alanine (Ala), valine (Val), isoleucine (Ile), and serine (Ser).

In the case of Gly, Ala, and Ser, they were obtained by boiling aqueous solutions of *ca* 0.01 mol of the aminoacid with an excess of basic copper carbonate for about half an hour. The excess of $CuCO_3.Cu(OH)_2$ was separated by filtration and the remaining, deep blue colored solutions, were concentrated over a water bath. The complexes precipitated by cooling the concentrated solutions [6].

Val and Ile complexes were prepared by dissolving 0.01 mol of the aminoacid in 10 ml of a 1.0 N NaOH solution and further addition of 25 ml of a 0.2 M $CuSO_4$ solution [6].

The obtained complexes were characterized by chemical analysis, infrared spectroscopy, and thermogravimetric studies. They respond to the following stoichiometries: $Cu(Gly)_2.H_2O$, $Cu(Ala)_2$, $Cu(Val)_2.H_2O$, $Cu(Ser)_2$, and $Cu(Ile)_2.H_2O$, respectively.

SOD assays

SOD activity was investigated by the method of Beauchamp and Fridovich [7] as improved by Imanari *et al.* [8,9]. This method is based on the inhibitory effect of SOD over the reduction of nitrobluetetrazolium (NBT) by the superoxide anion generated by the system xanthine/xanthine oxidase, at pH = 10.2 (carbonate buffer), measuring the absorption changes at 560 nm.

For comparative purposes we have also measured the activity of native superoxide dismutase from bovine erythrocites (Sigma), under the same experimental conditions.

All the regents used for the SOD assays were obtained from Sigma, and used as purchased.

Lipophilicity studies

For these assays the complexes were dissolved in physiologic solution and their copper concentrations determined by atomic absorption spectroscopy using a Perkin Elmer model 380 instrument with a lamp for multielement analysis.

A defined volume of these solutions was shaked with an equal volume of n-octanol. After the separation of both immiscible phases, the copper concentration

remaining in the aqueous layer was determined again by atomic absorption spectroscopy.

Values for the partition coefficients [10] were calculated from the determined concentrations.

RESULTS AND DISCUSSION

SOD-like activity

In Table 1 we present the results of the SOD assays performed with the five complexes, at different complex concentrations. In this table we show the percentage of inhibition of NBT reduction at any concentration. The 100% of superoxide activity corresponds to an assay performed in the absence of complex.

Complex	Concentration (M)	%-Inhibition
$Cu(Gly)_2.H_2O$	7.00×10^{-7}	7.39
	7.10×10^{-6}	14.64
	7.14×10^{-5}	22.53
	8.92×10^{-5}	32.50
	1.07×10^{-4}	96.40
	1.79×10^{-4}	97.19
	3.57×10^{-4}	98.48
	7.14×10^{-4}	98.55
$Cu(Ala)_2$	7.14×10^{-7}	5.53
	7.14×10^{-6}	14.79
	7.14×10^{-5}	26.72
	8.92×10^{-5}	98.09
	1.07×10^{-4}	98.19
	7.14×10^{-4}	98.66
$Cu(Val)_2.H_2O$	7.00×10^{-7}	3.31
	7.10×10^{-6}	3.89
	7.14×10^{-5}	18.87
	8.92×10^{-5}	65.87
	1.07×10^{-4}	98.54
	1.79×10^{-4}	98.54
	3.57×10^{-4}	99.42
$Cu(Ile)_2.H_2O$	7.00×10^{-7}	8.53
	7.10×10^{-6}	12.88
	7.14×10^{-5}	19.33
	8.92×10^{-5}	36.38
	1.07×10^{-4}	97.94
	1.79×10^{-4}	98.56
	7.14×10^{-4}	99.03
$Cu(Ser)_2$	4.43×10^{-7}	3.29
	4.43×10^{-6}	6.78
	4.43×10^{-5}	8.92
	6.64×10^{-5}	28.29
	1.11×10^{-4}	97.29
	2.21×10^{-4}	99.42
	4.43×10^{-4}	99.45

Table 1. Results of the SOD assays with the different complexes.

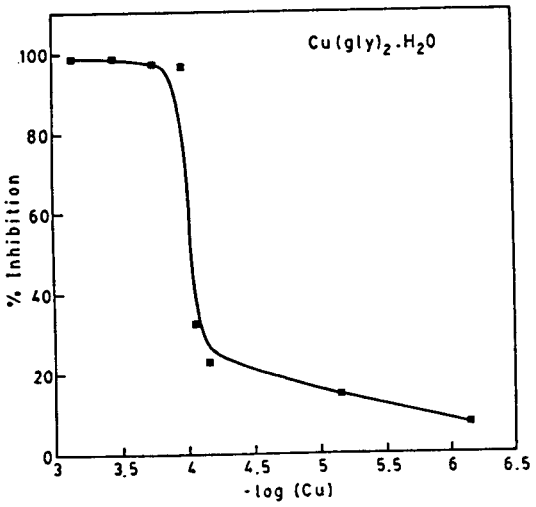

Figure 1. Percentage of inhibition of the NBT reduction as a function of the logarithm of Cu (II) concentration for the $Cu(Gly)_2.H_2O$ complex.

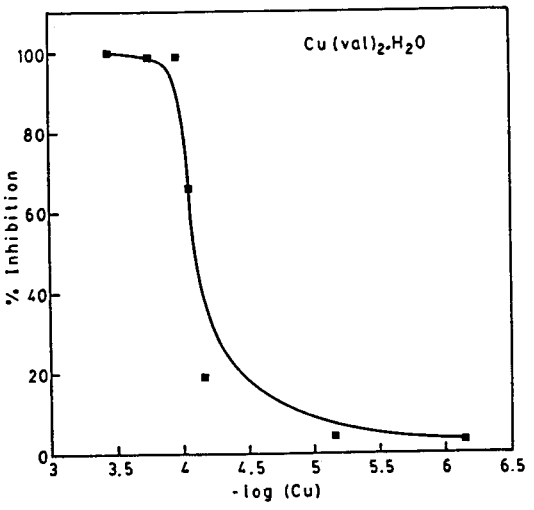

Figure 2. Percentage of inhibition of the NBT reduction as a function of the logarithm of Cu (II) concentration for the $Cu(Val)_2.H_2O$ complex.

In order to determine the concentration of complex required to yield 50% inhibition of the reaction, we plotted the percentage of inhibition against the logarithm of the metal concentration, as shown in Figures 1 and 2 for the glycine and valine complexes, respectively.

These plots allow a simple and rapid determination of the I_{50} values, which are summarized in Table 2. In this table we have also included the Cu(II) concentration of native SOD, which produces a 50% inhibition of the reaction (1.02×10^{-8} M) and have also compared the relations of concentrations ($[Cu]_{compl.}/[Cu]_{SOD}$) which yield 50% inhibition.

Although all the complexes present a significant activity, no clear trends on the effects of the different ligands on the SOD activity becomes evident from the data presented in Table 2. Therefore, it seems necessary to investigate the behaviour of a greater number of complexes of this type in order to attain a fuller understanding of this aspect.

Complex	Concentration (M)	$[Cu]_{compl.}/[Cu]_{SOD}$
$Cu(Gly)_2.H2O$	3.40×10^{-5}	3333
$Cu(Ala)_2$	3.23×10^{-5}	3166
$Cu(Val)_2.H_2O$	3.03×10^{-5}	2970
$Cu(Ile)_2.H_2O$	3.31×10^{-5}	3245
$Cu(Ser)_2$	3.72×10^{-5}	3647
Cu/SOD	1.02×10^{-8}	–

Table 2. Concentrations required to yield 50% inhibition.

On the other hand, we are also trying to correlate SOD-activity with other physicochemical parameters, such as the electrochemical behaviour of the complexes, their structural characteristics and the strength of the copper-ligand bonds.

Lipophilicity characteristics

It is currently assumed that partitioning between aqueous and organic phases serves as a model system of how biologically interesting solutes passes through membranes in living systems [10]. Therefore, we have determined the partition coefficients of four of the five complexes between physiologic solution and n-octanol. The serine complex could not be investigated due to solubility problems in both phases.

The results of these assays are shown in Table 3. The K-values are defined as the ratio of the Cu(II) concentration in the organic phase over its concentration in the aqueous phase.

Complex	sol. A	sol. B	K	log K
$Cu(Gly)_2.H_2O$	5.8	5.5	0.052 ± 0.015	-1.28
$Cu(Ala)_2$	6.1	5.7	0.066 ± 0.015	-1.18
$Cu(Val)_2.H_2O$	6.1	5.6	0.082 ± 0.010	-1.08
$Cu(Ile)_2.H_2O$	6.0	5.1	0.150 ± 0.010	-0.82

Table 3. Determination of the partition coefficient between physiologic solution and n-octanol. A: ppm Cu(II) in the original aqueous solution. B: ppm Cu(II) in the aqueous solution after contact with n-octanol.

The general trend observed in the tabulated K-values suggests that lipophilicity increases with ligand complexity. This trend follows a well known behaviour, according to wich in a homologous series the partition coefficients increase by added CH_2 group [10]. On the other hand, it is very interesting to comment that the found lipophilicity ordering follows that of the free aminoacids in n-octanol [10], but it is remarkably higher in the case of the copper-complexes, as can be seen from Table 4.

Aminoacid	log K (aminoacid)*	log K (complex)
Gly	-3.03	-1.28
Ala	-2.94	-1.18
Val	-2.10	-1.08
Ile	-1.71	-0.82

Table 4. Comparison of the partition coefficients (octanol/water) for the free aminoacids and their Cu(II) complexes.
* Values taken from Leo *et al.* [10]

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