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In order to characterize redox properties of biomolecules, specific methods for evaluating the antioxidant capacity of some biological structures or their components (like aminoacids) are used, such as chemiluminescence (CL) and cyclic voltametry coupled with UV-VIS spectroscopy. The CL antioxidant capacity, the reduction potential ($\varepsilon_{1/2}$), and the anodic current intensity (I_a) have been determined by controlling the behavior of the compounds under redox conditions.

The value of the half-wave potential of the first peak was correlated with the reduction tendency on the electrode, and this behavior was analyzed in relation to the antioxidant capacity observed by CL.

Keywords: aminoacids; chemiluminescence; cyclic voltametry; redox properties; UV-Vis spectroscopy

INTRODUCTION

Living cells, including those of humans, animals and plants, are continuously exposed to a variety of challenges that induce oxidative stress. This could stem from endogenous sources (normal physiological processes) and from exogenous sources, such as exposure to pollutants and irradiation (UV and ionizing) [1,2].

Oxidative stress leads the decrease in the antioxidant capacity of the system and is associated with the generation of reactive oxygen species including free radicals which are involved in the pathology of various diseases.

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Amongst the cell components, the proteins are also affected by the presence of the free radicals. As a result, it is useful to know their behavior in the presence of certain free radicals.

In this respect, some methods to evaluate the antioxidant capacity of various biological structures or their components, in this case chemiluminescence and cyclic voltametry are used [3,5].

In case of chemiluminescence (CL) the most popular system for light generation is the reaction of luminol with H_2O_2.

In the presence of H_2O_2 in a basic medium, the luminol is transformed in an excited dianion, and then it is desactivated by emitting light quanta at 430 nm. Introducing into the system some compounds of interest, the CL signal is amplified or diminished indicating a specific process of capture or release of short-live radical species that may be correlated with anti or pro-oxidative characteristics [6].

EXPERIMENTAL

The aminoacids selected for this study are representative for all their essential structures; moreover glutation and collagen were selected as proteins.

Apparatus

The chemiluminometer Turner Design TD20/20 (USA) at $\lambda = 430$ nm was used for determination of the chemiluminescence characteristics; the couple luminol (10^{-5} M solution in DMSO) – H_2O_2 (10^{-5} M solution in H_2O) in Tris–HCl (0.2 M) at pH = 8.6 have been used as standard. From the curve CL = f(t) the antioxidant activity and the rate constant have been determined.

In order to evaluate spectro-electrochemical characteristics a galvanostat–potentiostat Voltalab 21 system was used with a platinum electrode and a Ag/AgCl reference electrode in aprotic conditions. The support electrolyte was amonium tetraphyeniborate (TFBA) at $c = 10^{-1}$ M; the scanning speed was 10 mV/s for the catodic domain (0 and −1000 mV).

The UV-Vis spectrometer (Specord 2000–Germany) was coupled with the voltameter so that the spectrophotometer cell was also an electrochemical cell. In this way, the absorption spectra for the electrochemically generated chromophore species were recorded.

Solvents

DMSO (Fluka), and DMF (Merck).

RESULTS AND DISCUSSION

The CL characteristics of the aminoacids, the antioxidant activity of CL and the rate constant of first order reaction are presented in Table 1 [9].

The activity of the aminoacids was correlated with their acidity constants [7]. The glutation has an inhibiting activity similar to that of cysteine, both possessing –SH groups which, by subsequent oxidations, generate intermediaries with antioxidative capacity [8].

Under the conditions of the cyclic voltametry, the potential of the working electrode takes variable values depending on the direction and the scanning speed as a result of a succession of electrochemical processes (oxidations and reductions) of the ion-radical species generated on the electrode surface. By spectral analysis, various hipsochrome shifts are produced for some absorption bands after the end of the process; moreover, spectral modifications for some aminoacids were obtained during the catodic scanning consisting in the apparition of new absorption bands associated with the new species generated at the reduction potential peaks.

The carboxylic group is involved in the formation of the cation-radical, the species variants being interpreting according to the EC mechanism (Fig. 1).

TABLE 1 Chemiluminescent Characteristics of Aminoacids in Luminol + H_2O_2 System

Aminoacids		AA (%)	k_1 (s^{-1})
Glycine	Gly	52.0	0.010
Alanine	Ala	42.5	0.021
Valine	Val	49.5	0.013
Arginine	Arg	74.8	0.027
Leucine	Leu	39.0	0.014
Isoleucine	Ile	27.0	0.024
Lysine	Lys	56.4	0.049
Histidine	His	76.5	0.024
Tryptophan	Trp	48.0	0.011
Tyrosine	Tyr	72.0	0.055
Phenylalanine	Phe	60.6	0.045
Serine	Ser	66.0	0.046
Cysteine	Cys	99.3	0.034
Methionine	Met	60.2	0.038
Proline	Pro	62.0	0.038
Aspartic acid	Asp	58.7	0.028
Glutamic acid	Glu	55.0	0.017
Glutathion	–	99.5	0.073
Collagen	–	52.0	0.029

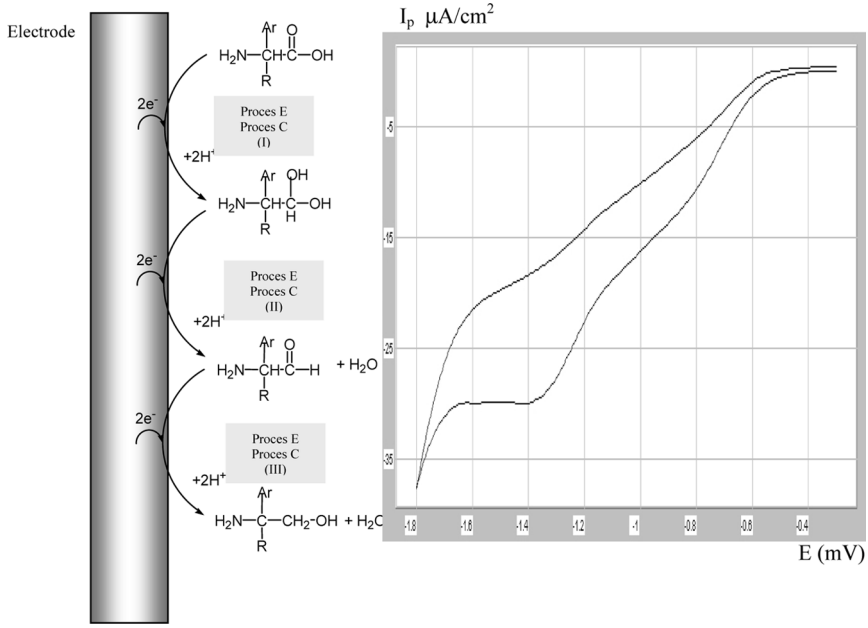


FIGURE 1 Possible mechanism of the cathod-coupled processes for tyrosine voltammogram.

The aminoacid voltammograms registered in -300 mV to -1800 mV domain show the presence of two reduction peaks which are much more evident than the oxidation peaks suggesting irreversible processes.

The size of the peak current, i_p, is directly proportional to the concentration of the aminoacid according to the Randles–Sevcik equation [3,10] with a linear form:

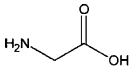
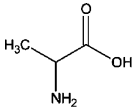
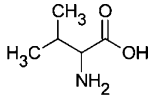
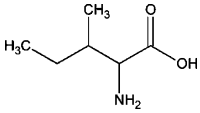
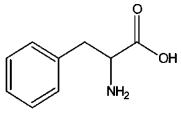
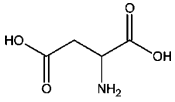
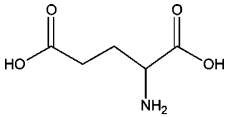
$$I_p = k \cdot c$$

where, k is a constant dependent on scanning speed, diffusion coefficient, surface of the electrode and number of electrons involved in the process.

In Table 2 the main electrochemical and spectral characteristics obtained from the investigation are presented.

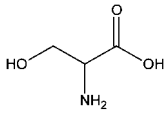
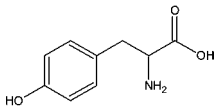
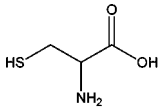
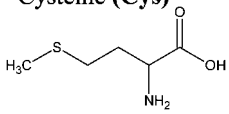
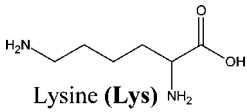
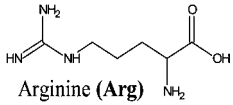
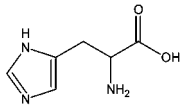
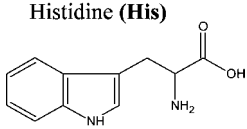
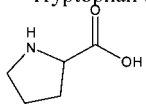
The value of the half-wave potential $\varepsilon_{1/2}$ (I) is associated with the reduction tendency on the platinum electrode of the aminoacid in order to form the anion-radical in the first electrochemical stage. The data indicate the triptophane is being the most active, while the phenylalanine is the least active among the aminoacids with an aromatic structure (Fig. 2).

TABLE 2 Spectro-Electrochemical Characteristics of Aminoacids

Aminoacid	Electrochemical data (reduction wave)				Spectral data	
	$\varepsilon_{1/2}$ (I) (mV)	$\varepsilon_{1/2}$ (II) (mV)	i_{pr} (I) ($\mu A/cm^2$)	i_{pr} (II) ($\mu A/cm^2$)	λ (nm)	Colour of anion radical
 Glycine **(Gly)**	−460	−690	97.65	125.43	–	–
 Alanine **(Ala)**	−450	–	32.28	–	–	–
 Valine **(Val)**	−450	–	154.23	–	–	–
 Isoleucine **(Ile)**	−395	–	225.21	–	–	–
 Phenylalanine **(Phe)**	−677	−1257	12.83	13.75	450	light yellow
 Aspartic Acid **(Asp)**	−350	–	108.32	–	–	–
 Glutamic Acid **(Glu)**	−380	–	335.24	–	–	–

(*Continued*)

TABLE 2 Continued

Aminoacid	Electrochemical data (reduction wave)				Spectral data	
	$\varepsilon_{1/2}$ (I) (mV)	$\varepsilon_{1/2}$ (II) (mV)	i_{pr} (I) ($\mu A/cm^2$)	i_{pr} (II) ($\mu A/cm^2$)	λ (nm)	Colour of anion radical
 Serine **(Ser)**	−320	–	280.57	–	440	Yellow-reddish
 Tyrosine **(Tyr)**	−402	–	63.28	–	–	–
 Cysteine **(Cys)**	−410	–	48.32	–	–	–
 Methionine **(Met)**	−412	–	75.34	–	–	–
 Lysine **(Lys)**	−650	–	180.42	–	440	Yellow-reddish
 Arginine **(Arg)**	−500	–	25.38	–	430	Light yellow
 Histidine **(His)**	−400	–	120	–	440	Yellow-greenish
 Tryptophan **(Trp)**	−420	−1420	15.50	12.42	500	Green-blue
 Proline **(Pro)**	−380	–	56.32	–	450	Light yellow

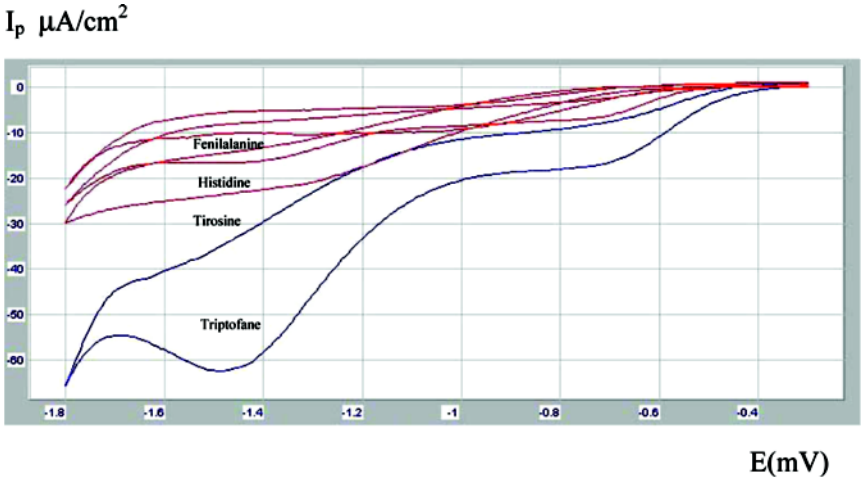


FIGURE 2 Cyclic voltammograms of the tryptophan, tyrosine, histidine, phenylalanine in DMSO solution.

These data allow to search if there is any correlation between the characteristics of the two methods. Thus, the $\varepsilon_{1/2}$ representation in function of the anti-oxidative activity of the aminoacids (Fig. 3) point

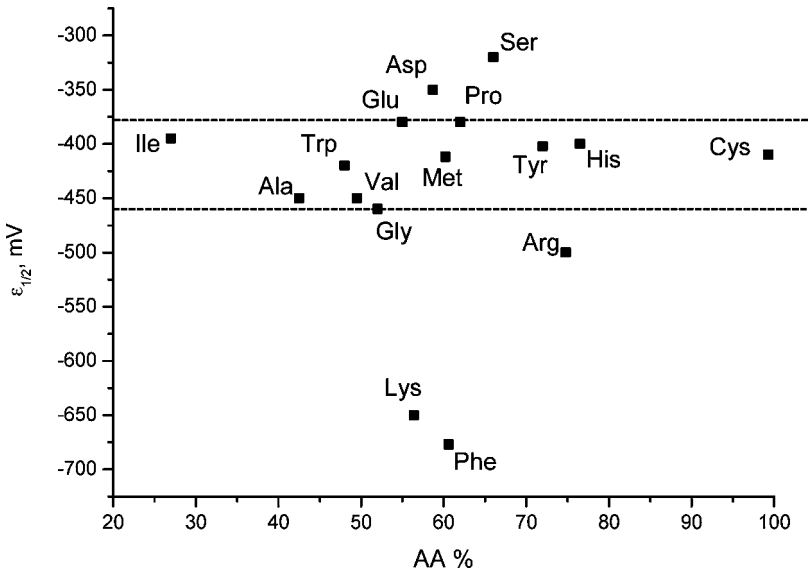


FIGURE 3 Variation of the half-wave potential versus the antioxidative activity.

out clustering of the majority of the biomolecules in the interval −375 −475 eV, with important deviations for lysine and phenylalanine. The relatively short above mentioned interval confirms that the reduction process takes place initially at the carboxylic group at low values of the potential in agreement with the high reactivity of these species. More electrochemically inert appear to be lysine and phenylalanine, the first with an aromatic group and the second with two amino groups separated by a long aliphatic chain.

The resistance to the oxidation in the CL system is related mainly to the chemical attack at the amino group explaining in this way the anti-oxidative character in a relatively wide domain, between 20 (isoleucine) and 99% (cysteine), even though there is a quite constant activation energy in the process of electrochemical reduction. The lowest antioxidative activity of isoleucine may be attributed to its less reactive structure, while the high antioxidative activity of cysteine is explained by the presence of the reactive group SH, that leads by oxidation to products with a pronounced inhibitory activity.

A similar behavior is shown in Figure 4 for the variation $\varepsilon_{1/2}$ against the rate constant of the oxidation reaction in the

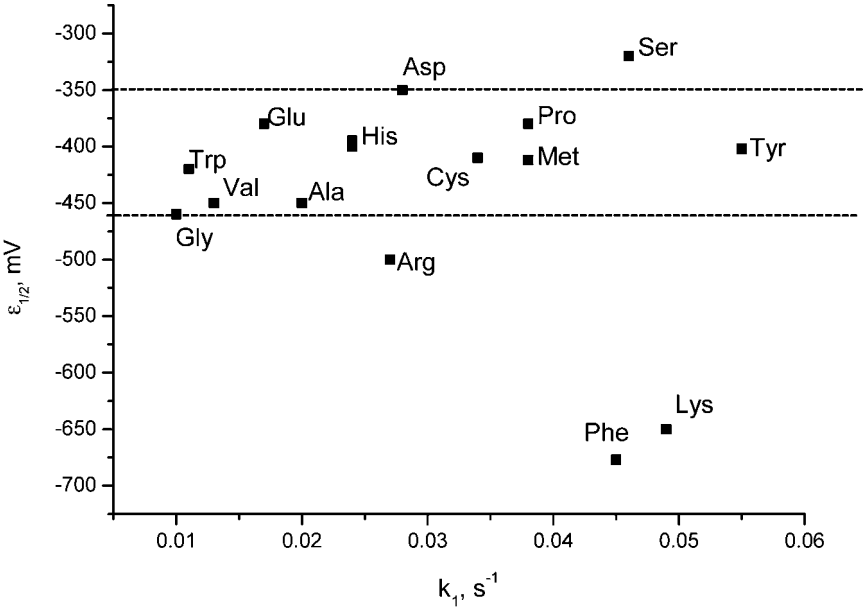


FIGURE 4 Variation of the half-wave potential versus the reaction rate constant.

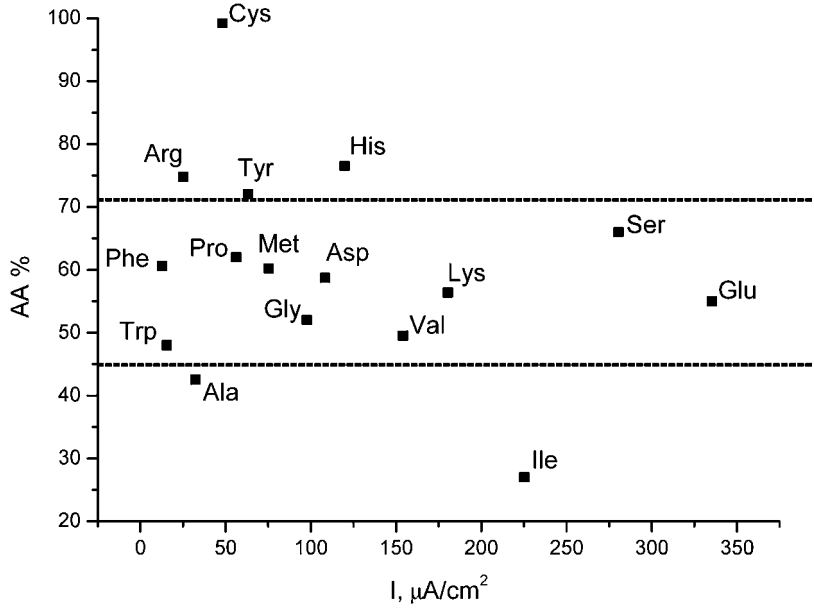


FIGURE 5 Variation of the anti-oxidative activity with the intensity of the peak current.

chemiluminescence process. In this case once again major deviations of the lysine and phenylalanine are noticed, while the increasing sequence of the rate constant is reverse in relation to the increase in the antioxidative activity (see Fig. 3), according to the antagonic effect of the two parameters.

The majority of the aminoacids with a value of the antioxidative activity ranging between 50–70% show a variable peak current (i_I) (Fig. 5). Isoleucine has the lowest antioxidative activity, while cysteine presents a maximal antioxidant activity caused by the sulfhydric group, as it was already mentioned.

Representation of free energy relation between kinetic data of CL and acidity constants of aminoacids (Fig. 6) reveals a quite linear dependence for most of biomolecules, significant deviations being noticed for arginine, histidine and aspartic acid.

The ensemble of these data outlines the complexity of the mechanisms taking place in electrochemical conditions, a correlation with the processes carried out by chemiluminescence, respectively with the antioxidative activity and kinetic data being note straightforward.

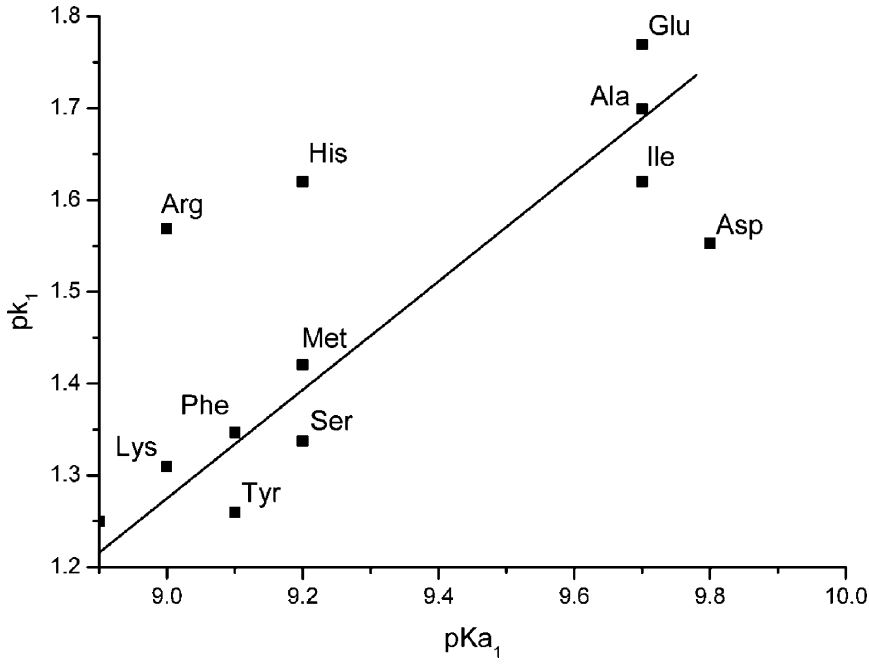


FIGURE 6 Free energy relation.

These results can be extended on glutation and collagen, which are biomolecules with complex proteic structures. In this case the redox processes involved in the two methods occur in a similar maner and on the same functional groups.

CONCLUSION

The main redox characteristics of some essential aminoacids were determined by chemiluminescence and spectro-electrochemical procedures.

The special complexity of the electrochemical process at cathode was noted, since its characteristics – the half-wave potential ($\varepsilon_{1/2}$) and the current of the first peak ($i_{p(I)}$) cannot be directly correlated with the antioxidative activity and kinetic data determined by chemiluminescence. Such a poor correlation could by interpreted be the different mechanisms involved in the two techniques, as the electrochemical reduction has as main effect the decarboxilation process, while the antioxidative activity established by CL is determined in essence by the attack at the amino group.

For other groups of biomolecules in which the two redox processes are performend at the same functional group, a better correlation of the parameters is expected for the two methods.

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