KINETICS OF OXIDATION OF AMINO ACIDS BY SOME FREE STABLE HYDRAZYL RADICALS‡

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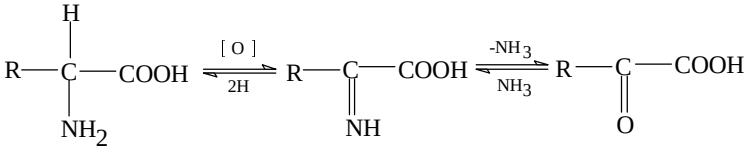
Abstract

The kinetics of amino acids (Ser, Pro, Leu, Trp, Thr, Phe, Met, His) oxidation by sodium salts of 2-*p*-phenylsulfonic acid-2-phenyl-1-picrylhydrazyl (**I**) and 2,2-di-*p*-phenylsulfonic acid-2-phenyl-1-picrylhydrazyl (**II**) at isoelectric point of amino acids has been studied over the temperature range 298 - 318 K. The rate studies were made under pseudo-first order conditions with an excess of amino acid over the oxidant. The kinetics was followed by monitoring the disappearance of **I** and **II**, spectrophotometrically, at 520 nm and 514 nm, respectively. The activation parameters were determined from rate constant dependence on temperature. The amino acids with aromatic structure (His, Trp, Phe) were oxidised more rapidly than the others. A mechanistic pathway for amino acids oxidation was proposed and discussed, similarly with their enzymatic degradation, which have as final products α–keto-acids and ammonia. In this aim was followed a mechanistic and structural investigation grounded on isokinetic theory.

Keywords: kinetics; free radical; hydrazyl; amino acids; BSA

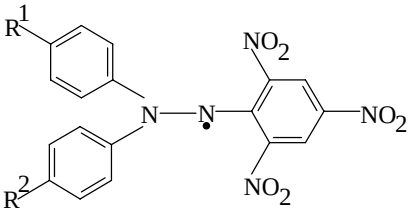
Introduction

Amino acids represent for organism forerunners of essential biomolecules such as proteins, hormones, enzymes, etc; also, they may serve as energy source, losing their amino group by two pathways: transamination or oxidative deamination. The steps of oxidative deamination are resumed in the following scheme [1-3]:



Scheme 1. Oxidative deamination of amino acids

The objective of the present paper is the kinetic study of oxidative deamination of amino acids, using two free stable water soluble radicals of hydrazyl type, namely sodium salts of 2-(*p*-phenylsulphonic acid)-2-pheny-1-picrylhydrazyl $NaSO_3DPPH^{\cdot}$ and 2,2-(*p*-phenylsulphonic acid)-1-picrylhydrazyl $(NaSO_3)_2DPPH^{\cdot}$, respectively (Fig. 1).



I $R^1 = NaSO_3$ $R^2 = H$ $NaSO_3DPPH^{\cdot}$

II $R^1 = R^2 = NaSO_3$ $(NaSO_3)_2DPPH^{\cdot}$

Fig. 1. The structure of the free stable radicals employed.

Such radicals are oxidising species, and owing to their properties they have been applied in quantitative determinations of amines, phenols, vitamins [4-6]. The ESR spectra of both radicals consist in a broad quintet with two practically equal hyperfine coupling constants $a_N \cong 9.0$ G [7].

Isokinetic theory is a diagnostic tool in the field of structural and mechanistic investigation [8]. In this theory, the intersection point of Arrhenius lines for a reaction series is equivalent with a linear relationship between activation energy and preexponential factor or between entropy and enthalpy terms [8]. In this paper the isokinetic theory was applied in order to elucidate the mechanistic pathway.

Experimental

Substances. The free radicals $NaSO_3DPPH^{\cdot}$ and $(NaSO_3)_2DPPH^{\cdot}$ were obtained by method described in literature [7, 9]. Amino acids of L-configuration were Loba Chemie products.

Methods and apparature. Kinetic measurements were made in aqueous solution at 298-318 K, with an excess of amino acid over the free radical (about 100 times; final concentration of both radicals were 10^{-4} M ± 5% and final concentration of amino acids were 10^{-2} M). The kinetics was followed by monitoring the disappearance of $NaSO_3DPPH^{\cdot}$ (or $(NaSO_3)_2DPPH^{\cdot}$) at λ_{max} = 520 nm (or 514 nm, respectively); at this

wavelength the absorption of the amino acids or the corresponding hydrazines derived from hydrazyl radicals were negligible. The measurement were carried out with an SPECORD M40 apparatus. The course of the reaction was studied for al least two half-live, and the rate constant were evaluated from linear plots of absorbance against time. The solution of amino acids and free radicals were separately thermostatted and then mixed in equal volumes; during the measurements the temperature was mentioned at the chosen value. The pseudo-first order rate constant calculated were reproducible to within 4%. Variation of the ionic strength of the reaction mixture by adding NaCl (within 0.1 M concentration) had no effect on the rate of the reaction in both free radicals employed.

Product analysis. The reaction mixture were worked up, after 24 hrs., as follow: i) an acidic solution of 2,4-dinitrophenylhydrazine was added, and after 24 hrs. a red precipitate appear, involving the presence of α-keto-acids [1, 2]; ii) ammonia was identified with Nessler reactive; iii) by UV-VIS analysis presence of the corresponding hydrazine of the free radicals were confirmed (λ_{max} = 350 nm) [7].

The redox potentials of free radicals were determined the same procedure described elsewere [10, 11]; experimental conditions: Princeton Applied Research Potentiostat 273A with an IBM PS2 acquisition system; stationary cyclic voltammetry technique with Pt electrodes (wire, 0.2 cm^2 area) as working and auxiliary electrodes, and SCE as reference electrode; scan rate 0.05-0.5 V/s; concentration of compounds 10^{-3} M).

Results and Discussion

Despite these radicals are called “stable” owing to their great stability in time (years) [9], “persistent” may be better employed, because our results show that even in aqueous solution, the free stable radicals undergo a slow decomposition; thus, disappearance of both free radicals is the result of two parallel processes, as shown ours attempts:

$$NaSO_3DPPH^{\cdot} + RCH(NH_2)COOH \xrightarrow{k_1} \text{products} \qquad (1)$$

$$NaSO_3DPPH^{\cdot} + HOH \xrightarrow{k_1'} \text{products} \qquad (2)$$

The same reactions can be written for the $(NaSO_3)_2DPPH^{\cdot}$ free radical (with kinetic constants k_2 and k_2', of course); so the experimental kinetic constant is $k_{exp} = k_1 + k_1'$, where k_1' was determined in a separate experiment ($k_1' = 0.76 * 10^{-5}\ s^{-1}$, $k_2' = 1.24 * 10^{-5}\ s^{-1}$). The influence of the pH over these kinetic constants were small; thus, in the 5-8 units pH range the variation was smaller then 10%; also, it is well known that another hydrazyl free radical, DPPH (2,2-diphenyl-1-picrylhydrazyl), is stable at 6-8 area of pH only [12]. On notice that the compound **I** and **II** have similar reactivity. Table 1 shown the kinetic constant determined for amino acids oxidation by free radicals **I** and **II**.

Table 1. Kinetic constant for amino acid oxidation at 306 K by $NaSO_3DPPH^{\cdot}$ (k_1) or $(NaSO_3)_2DPPH^{\cdot}$ (k_2) and the correlation coefficient *r*.

Amino acid	*p*I[13]	k_1 x 10^5 (s^{-1})	r_1	k_2 x 10^5 (s^{-1})	r_2
Phe	5.91	3.21	0.989	2.29	0.989
His	7.64	9.40	0.992	6.62	0.981
Leu	6.04	1.20	0.998	1.43	0.988
Met	5.74	3.57	0.999	2.77	0.986
Pro	6.30	1.19	0.989	1.76	0.986
Ser	5.68	1.82	0.994	2.17	0.989
Thr	5.86	2.00	0.987	1.78	0.996
Trp	5.88	13.00	0.990	6.74	0.982

In many cases, the rate constants k_1 and k_2 are close, except values for Trp, His, and Phe. For these amino acids, the aromatic structure of the R moiety ($RCH(NH_2)COOH$) and moreover the presence of a supplementary nitrogen atom for Trp and His can influence the reactivity of amino acids [1, 2].

In order to find out the redox potential of the compounds **I** and **II**, cyclic voltammetry was performed for the redox couple S-$DPPH^-$ (anion) / S-$DPPH^{\cdot}$ (radical), where S stands for SO_3Na or $(SO_3Na)_2$, eq. 3.

$$S\text{-}DPPH^{\cdot} + e^{-} \rightleftharpoons S\text{-}DPPH^{-} \quad (3)$$

The experimental oxidation potentials obtained were 1.667 V and 1.576 V, respectively. As was state above, the reactivity of these compounds are comparable.

The rate constant dependence upon temperature was also studied. Using Arrhenius equation the activation parameters were determined for each amino acid - free radical pair (eq. 4-6) [8].

$$k = Ae^{-E_a/RT} \quad (4)$$

$$A = e\frac{kT}{h}e^{\Delta S^*/R} \quad (5)$$

$$E_a = \Delta H^* + RT \quad (6)$$

The activation parameters obtained from Arrhenius lines are shown in Table 2 and Table 3. The results shown in these tables may explain the variation of the rate constants (see also Table 1).

Table 2. Activation parameters from amino acids oxidation by free stable radical **I**

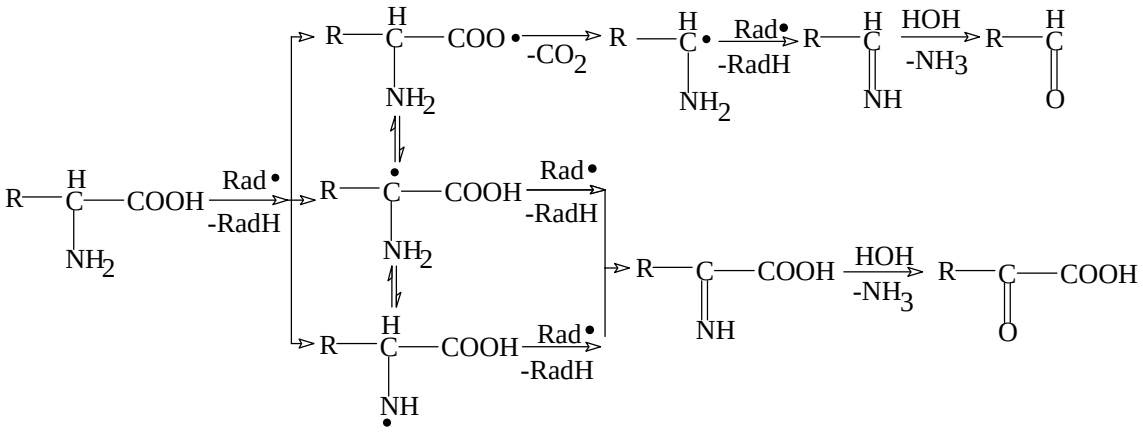
Amino acid	E_a (kJ/mol)	lnA	ΔS^* (J/molK)	ΔH^* (kJ/mol)
Fen	81.88	23.744	-55.85	79.387
His	9.32	-4.503	-290.54	6.827
Leu	42.12	-3.835	-285.00	39.62
Met	108.22	33.193	22.70	105.72
Pro	18.36	-1.166	-262.81	15.86
Ser	20.65	-0.426	-256.67	18.15
Thr	21.105	-0.353	-256.06	18.61
Trp	14.21	11.00	-161.05	11.71

Table 3. Activation parameters from amino acids oxidation by free stable radical **II**

Amino acid	E_a (kJ/mol)	lnA	ΔS^* (J/(molK)	ΔH^* (kJ/mol)
Fen	60.41	14.97	-128.55	57.94
His	11.96	4.05	-286.61	9.49

Leu	73.62	18.39	-100.10	71.15
Met	37.00	5.44	-207.75	34.53
Pro	49.86	9.78	-171.60	47.39
Ser	37.89	5.48	-207.40	35.42
Thr	68.22	16.84	-113.01	65.75
Trp	31.74	4.85	-212.60	29.27

The activation energy E_a is smallest for His, Thr and Trp, explaining the higher oxidation rate of these amino acids. An oxidation mechanism of amino acids by free radicals $NaSO_3DPPH^{\cdot}$ and $(NaSO_3)_2DPPH^{\cdot}$ is proposed in Scheme 2.



Scheme 2. Pathway of oxidation of amino acids by free hydrazyl radicals (noted as Rad).

Scheme 2 shown that in a first step it is possible to obtain three types of amino acid radicals, with the unpaired electron on oxygen, carbon or nitrogen, the second one being probably more stable. These radicals are achieved by a hydrogen atom transfer between amino acid and $NaSO_3DPPH^{\cdot}$ or $(NaSO_3)_2DPPH^{\cdot}$ radical. In the first case the oxidative deamination of amino acid occurs, with evolution of ammonia and aldehyde (which can be further oxidised to acid). In the second and the third cases the initially formed amino acid radical is further oxidised to the imino acid, which in water presence yields ammonia and keto acid, as it was presented in experimental part. The hydrazones identified by theirs melting point derived only from the corresponding α-keto acids, but also some unidentified compounds of red colour were observed. We do not exclude first pathway, because hydrazyl radicals are known as a good hydrogen abstractor from

amines [4]. The oxidation of some amino acids by Fremy's salt (potassium nitrosodisulfonate) or by alkaline hexacyanoferrate (III) unfold in this way [1, 2]. For His and Trp the nitrogen from R moiety can be also involved in such processes. The attempt to trapped the short-lived free radicals (see Scheme 1) with a spin-trapper (*t*-butyl-α-phenylnitrone or 2-methyl-2-nitrosopropane) at room temperature failed; only at 77 K this kind of radicals could be evidenced, as literature data indicated [1].

Some isokinetic aspects. Isokinetic theory was applied in this paper for mechanistic investigation. One of the approximation made in results interpretation was the attempt to apply the active complex theory for the obtaining of the kinetic parameter from oxidation of amino acids [8, 14, 15]. The linear correlation between rate of the reaction at different temperature shown that a common point of intercept does not exist between Arrhenius lines, which means that an effect of compensation between activation entropy and enthalpy does not exist, however by plotting ΔH^* versus ΔS^* for both series of reactions it is possible to achieve an apparent linear relationship (see Fig. 2, *r* = correlation coefficient) [16].

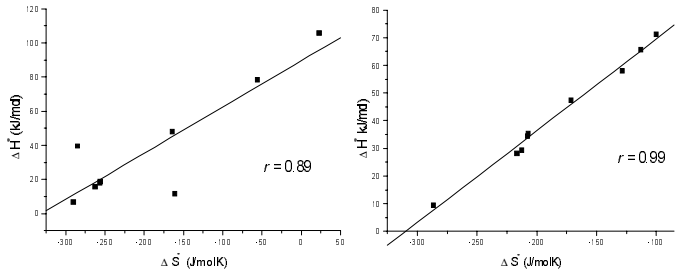


Fig. 2. Plot of ΔH^* versus ΔS^* for the compound **I** and **II**

The correct representation between activation parameters ΔH^* and ΔG^* ($\Delta G^* = \Delta H^* - T\Delta S^*$) shown the absence of some compensation effect between ΔH^* and ΔS^*, which means that derived parameters ΔH^* and ΔS^* are statistically dependent which cannot be used for further regression analysis in aims to find any isokinetic relationship (Fig. 3) [8, 16].

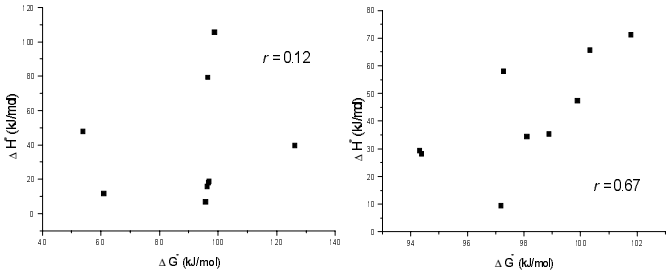


Fig. 3. Plot of ΔH^* versus ΔG^* for the compound **I** and **II**

In conclusion, analysis of the obtained results can supply information about the mode of action on reaction conditions, for changing the factors which determine the active complex formation. The mechanistic pathway of amino acids oxidation occurs probably by an intermediate of amino acid radical type, which leads further to keto acid. This study presents also some importance in understanding of natural ageing and oxidative stress processes (mainly due to the generation of free radicals *in vivo*).

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Povzetek

Raziskali smo kinetiko oksidacijo amino kislin pri izoelektrični točki in temperaturah med 298 do 318 K. Reakcije smo zasledovali spektrofotometrično pri 520 in 514 nm, odvisno od uporablčjenega oksidanta. Ugotovili smo, da oksidacija amino kislin z aromatskimi obroči (His, Trp, Phe) poteka hitreje kot ostalih. Predlagamo možen mehanizem oksidacije

amino kislin, po katerem pdobno, kot pri encimatski razgradnji, nastane α-keto kislina in amoniak.