Journal of Inclusion Phenomena and Macrocyclic Chemistry **39:** 269–271, 2001.

Influence of Cyclodextrins on the Kinetics of Oxidation of Amino Acids and BSA by Hydrazyl Radicals

GABRIELA IONITA[1], PETRE IONITA[2], VICTOR EM. SAHINI[3] and CONSTANTIN LUCA[4]
[1]*University of Pitesti, Dept. of Physical Chemistry, Gh. Doja Street 41, 0300 Pitesti, Romania;* [2]*Institute of Physical Chemistry, Spl. Independentei 202, Bucharest, Romania;* [3]*University of Bucharest, Faculty of Chemistry, Bd. Carol I 13, 70346 Bucharest, Romania;* [4] *"Politehnica" University Bucharest, Department of Analytical Chemistry, Polizu 1, Romania*

(Received: 24 March 2000; in final form: 22 May 2000)

Key words: kinetics, hydrazyl, cyclodextrin, amino acids, BSA

Abstract

The kinetics of oxidation of amino acids (Arg, His, Lys, Phe, Thr and Tyr), a dipeptide (Gly-His), and BSA (bovine serum albumin) by two persistent water soluble free radicals of the hydrazyl type has been studied. The rate decreases in the order Arg>Lys>Tyr>Thr>His∼BSA∼Phe∼Gly-His with both free radicals. Addition to the reaction mixture of α- and β-cyclodextrin decreases the oxidation rate, probably due to amino acid encapsulation in the cyclodextrin cavity. β-Cyclodextrin protects more efficiently against oxidation than α-cyclodextrin.

Introduction

Previous work has shown that the oxidation of amino acids by sodium salts of 2-*p*-phenylsulfonic-acid-2-phenyl-1-picrylhydrazyl (**1**) and 2,2-di-*p*-phenylsulfonic-acid-1-picrylhydrazyl (**2**) is easily monitored spectrophotometrically by the disappearance of **1** or **2** at 515 nm [1]. Amino acids are oxidized to α-keto-acids (Figure 1), similar to the reaction occuring during enzymatic oxidation (desamination).

The ability of cyclodextrins to form inclusion complexes in solution is well known, due to a toroidal shaped cavity. α- and β-Cyclodextrins are cyclic oligomers composed of six or seven α-D-glucopyranose rings connected by 1,4′-O-glycosidic bonds [2]. The dimension of the cavity-shaped binding site is 4.7–5.3 Å for α- and 6–6.5 Å for β-cyclodextrin; the depth of their cavities is 7.9–8.0 Å [3, 4]. Cyclodextrins act as receptors for organic substrates, including amino acids and their derivatives [5, 6]; cyclodextrins are also used in supramolecular catalysis [7].

This paper deals with a kinetic study of the oxidation of amino acids and bovine serum albumin (BSA) by the free radicals **1** or **2**, in the absence or presence of α- or β-cyclodextrin. The aim is to show that cyclodextrins can protect some bioactive compounds against free radical oxidation (it is known that in the process of ageing the presence *in vivo* of free radicals increases); also, oxidative stress is responsible for the generation of free radicals.

Experimental

Substances. Amino acids of L-configuration were from Loba-Chemie. BSA was from Merck. The persistent free radicals **1** and **2** were obtained as described in the literature [8]. α- and β-Cyclodextrins were from Aldrich and dextrin from Serva.

Methods and apparatus. Kinetic measurements were made in aqueous solution at 298 K, with a large excess of amino acids and cyclodextrin over free radical (final concentrations: free radical 10^{-4}M; amino acid 10^{-2}M; cyclodextrin 2×10^{-2}M). The kinetics was followed by monitoring the disappearance of **1** or **2** at 515 nm; at this wavelength the absorbance of the amino acids and BSA or of other reaction products were negligible. The measurements were carried out with a Specord M40 spectrometer. The measurements were made for two half lives, and the rate constants were evaluated from linear plots of logarithm of absorbance against time. The solution of amino acids, free radical and cyclodextrins were separately thermostated and then mixed in equals volumes; during the measurements the temperature was maintained at 298 ($\pm$ 0.2) K. The pseudofirst order rate constants calculated were reproducible within 5%.

Results and discussion

The free radicals **1** and **2** undergo a slow decomposition in aqueous solution, as was shown in a previous paper [1]. Preliminary experiments showed that in the presence of cyclodextrin the decomposition is slowly accelered (the rate constant of decomposition in water of the free radicals are 0.028 min^{-1} and 0.065 min^{-1}, respectively). In the presence of α- or β-cyclodextrin the kinetic constants are 0.041 $min^{-1}$and 0.083 min^{-1}. For the determination of the kinetic constant of amino acid oxidation these constants were taken into account.

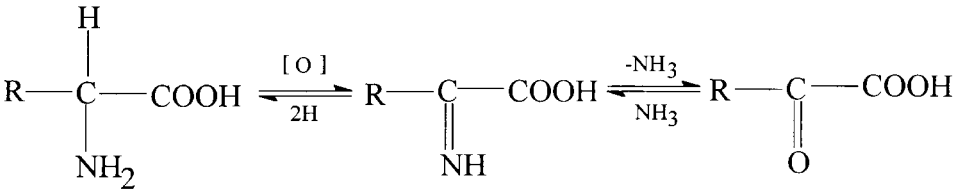

Figure 1. Oxidative desamination of amino acids.

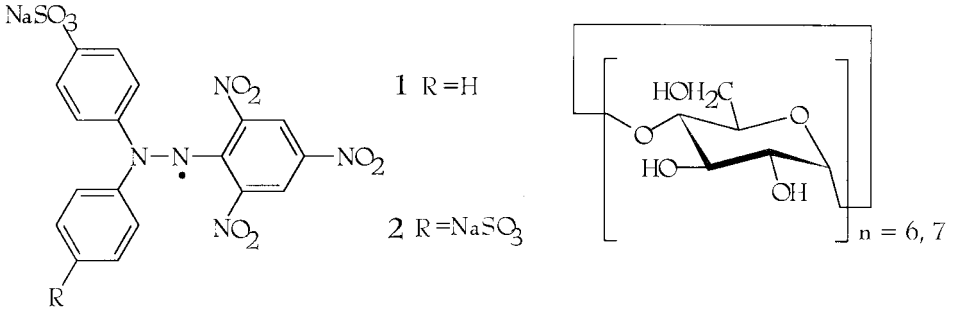

Figure 2. Structures of free radicals **1** and **2** and of α- and β-cyclodextrin (n = 6 or 7, respectively).

Table 1. Rate constants ($\times 10^3$ min^{-1}) obtained in the absence (k_n) or presence of α- (k_n^{α}) and β-cyclodextrins (k_n^{β}) using free radical **1** (n = 1) or **2** (n = 2)

Amino acid	k_1	k_1^{α}	k_1^{β}	k_2	k_2^{α}	k_2^{β}
Arg.HCl	0.34	0.32	0.28	0.74	0.62	0.48
BSA	0.65	0.60	0.30	0.95	0.92	0.80
His	0.52	0.44	0.41	1.47	1.45	1.05
Lys	2.65	2.49	1.91	2.37	2.37	2.25
Gly-His	0.40	0.37	0.36	0.38	0.23	0.24
Phe	0.39	0.36	0.33	1.38	1.19	0.93
Thr	1.53	1.56	0.51	5.41	5.18	4.03
Tyr	2.17	2.04	1.01	7.65	7.32	6.06

The reaction of free radicals **1** and **2** with Arg is so fast that it was not possible to determine the rate constant. Using the hydrochloric acid salt Arg.HCl the rate constant could be determined, because the *p*I (isoelectric point) of the amino acids (*p*I Arg = 10.8) plays an important role in their oxidation [9, 10]. Table 1 shows the rate constant (*k)* values obtained from amino acids, BSA and Gly-His peptide.

Lys also reacts fast (*p*I = 9.7), followed by Tyr. The increased reactivity of Tyr may be explained by the presence of a phenolic group. Literature data shows that hydrazyl free radicals react easily with phenols and thiols [11]. The kinetic constants include the supplementary oxidation of the -OH group. Previous work has shown that amino acids with an aromatic moiety are usually oxidized faster that the others [1]. Addition of α- or β-cyclodextrin decreases the oxidation rate, β- cyclodextrin being the more efficient in the inhibition of the oxidative process.

Table 1 shows the rate constants obtained using radical **2**. The rate constants are higher, due to the increased reactivity of **2**. The amino acids used are the same, and the use of cyclodextrins in the oxidation process decreases the rate constant (reaction occurs more slowly). β-Cyclodextrin seems also to be efficient in protection against oxidation.

The dipeptide and BSA were used to see if their oxidation rates are close to those of the other amino acids and also to see if the cyclodextrins decrease the oxidation rate. For the dipeptide, the rate constant for both free radicals are similar and small. The addition of cyclodextrin decreases the oxidation rate only very slightly. With BSA, the rates are different for the free radicals **1** and **2** (higher for **2**, due to higher reactivity), and cyclodextrin decreases the rate of oxidation.

Regarding the mechanism of the oxidation process, previous papers have shown that the α-keto-amino acid is the oxidation product of amino acids [1, 9, 10]. The oxidation of amino acids with other persistent free radicals (Fremy's salt, potassium nitrosodisulfonate) shows the same results [9]. In order to investigate the mechanism of oxidation inhibition by cyclodextrin, a separate experiment was performed using plain dextrin. The results show that oxidation inhibition does not occur or is very weak; this indicates that the *cyclic structure* of α- and β- cyclodextrins is responsible for the oxidation inhibition. Amino acids and some derivatives give inclusion complexes with cyclodextrin; β-cyclodextrin usually forms more stable complexes, owing to its size which is more suitable for the shape and dimension of the R moiety of amino acids ($RCHNH_2COOH$) [2, 4, 5]. The binding constants are small in the case of aliphatic amino acids, but higher for aromatic amino acids.

In conclusion, amino acid and protein oxidation is inhibited by the presence of cyclodextrins; thus, cyclodextrins are able to protect amino acids and proteins against oxidation, and this is indirect evidence of the encapsulation of amino acids in the cyclodextrin cavity. We do not exclude also the possibility that compounds **1** and **2** form inclusion complexes with α- and β-cyclodextrins, but our separate study [1] proved that the reactivity of these free radicals is almost independent of the presence or absence of cyclodextrins.

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