Gamma-Radiolysis of Aqueous Solution of Histidine

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The dependence of $G_i(-M)$ values of histidine on the concentration of irradiated $10^{-4}-10^{-1}$ M solutions, pH and the presence of O_2, N_2O and *sec*-butyl alcohol was investigated. In oxygen-free medium the maximum radiation sensitivity of histidine was found at pH 5–8; in oxygenated solutions it was shifted to the 6–11 pH range. The formation of radiation products was also studied. The course of radiation decomposition of histidine depends on the presence of oxygen and on the pH of irradiated solution.

In comparison with other amino acids histidine belongs among substances that are considerably labile to radiation [1, 2]. Its high reactivity with OH radicals and hydrated electrons has been demonstrated on the basis of the results of pulse radiolysis by Braams [3] and also Rao, Simic and Hayon [4].

However, more detailed informations on the course of radiolysis of histidine and on the formation of radiation products have not been available so far. Some data were obtained on photolysis of histidine [5–9]. In the UV-irradiated solutions not only substances with intact imidazole nucleus as imidazolyllactic and imidazolylacetic acids were proved, but also substances formed by the splitting off of this nucleus (α-alanine, glycine, serine) and products formed by its cleavage (aspartic, glutamic and OH-glutamic acids, asparagine, β-alanine and proline).

The aim of this study was to obtain more detailed informations on the changes taking place in aqueous solutions of histidine, irradiated by ionizing radiation, where attention was concentrated

1.) on the evalution of the initial radiation loss of histidine, irradiated in solutions of various concentrations, within a broad range of pH, and in the presence of some radical scavengers,
2.) on the identification of the main products of radiolysis of histidine.

Experimental

For the preparation of solutions A grade histidine (Calbiochem) and redestilled water were used; in some instances [U-^{14}C]histidine (ÚVVVR, Prague) and L-[^{14}C]histidine (Amersham, the Radiochemical Centre) was also added. The irradiated aqueous solutions of histidine were in the $5\times10^{-4}-10^{-1}$ M concentration range. The irradiation was carried out both in solutions bubbled through with oxygen during the operation, and in solutions which were bubbled through with nitrogen suboxide or nitrogen before irradiation. To some of these samples *sec*-butyl alcohol (resulting concentration 1 M) was also added. The pH of the solutions was adjusted by addition of A grade sulfuric acid and NaOH.

^{60}Co was used as a radiation source, the intensity was 4×10^{18} eV/ml/h. The samples were irradiated with doses from 13×10^{17} to 13.4×10^{20} eV/ml. The determination of histidine and its radiation products was carried out both radiometrically and colorometrically, using a quantitative test with ninhydrine [10]. The separation of histidine from the radiation products was carried out mainly by paper electrophoresis (Whatman N. 3, pyridine-acetate buffer pH 5.6, 1400 V). In some cases, the electrophoresis was combined with paper chromatography in the system *n*-butyl alcohol – acetic acid – water (4 : 1 : 5). For qualitative tests ninhydrin, Pauly's reagent, acid-base indicator and 2,4-dinitrophenylhydrazine were employed.

The determination of the total content of amides and free ammonia was carried out by combined microdiffusion methods according to Conway [11]. The recombination products of histidine were isolated from the irradiated solutions using Sephadex G-10 (column height 46 cm, diameter 1.1 cm; the elution was carried out with 0.01 N acetic acid). The molecular mass of the recombination products was estimated by comparing their elution volumes with those of reduced and oxydized glutathione and pilocarpin.

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Results and Discussion

Figs 1 and 2 represent the decrease of histidine irradiated in neutral solutions of various concentrations, using graded doses of radiation, under saturation with oxygen and in its absence. The dependence of the loss of histidine on the dose is characterized by a series of straight lines the slopes of which are indirectly proportional to the concentration of the irradiated solution. From the slope of these curves $D/50$ were read and the initial radiation decreases of histidine $G_i(-M)$ were computed. The determined values of the initial decreases in variously concentrated histidine solutions are given in Table I.

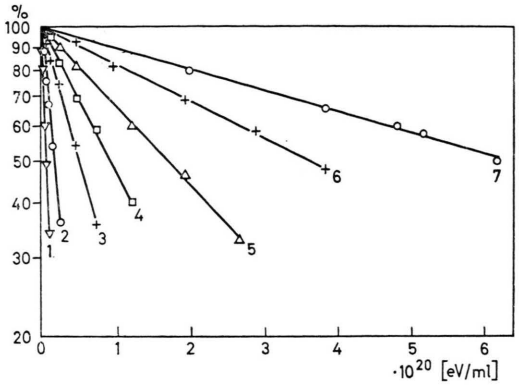

Fig. 1. Losses of histidine irradiated in neutral aqueous solution under oxygen in dependence on the absorbed dose. The initial concentration of histidine [M]: 1— 5×10^{-4}; 2— 10^{-3}; 3— 5×10^{-3}; 4— 10^{-2}; 5— 2×10^{-2}; 6— 5×10^{-2}; 7— 10^{-1}.

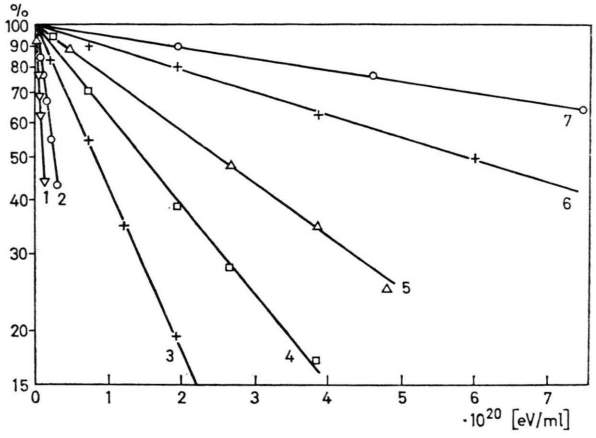

Fig. 2. Losses of histidine irradiated in neutral aqueous solution under nitrogen in dependence on the absorbed dose. The initial concentration of histidine [M]: 1— 5×10^{-4}; 2— 10^{-3}; 3— 5×10^{-3}; 4— 10^{-2}; 5— 2×10^{-2}; 6— 5×10^{-2}; 7— 10^{-1}.

From Table I it follows that during the irradiation of histidine in oxygen-free medium its $G_i(-M)$ values gradually increase from the region of the lowest concentrations up to the 10^{-2} and 10^{-1} M concentrations, when a certain stabilization of the radiation decrease is attained about the value of 3.5. In solutions saturated with oxygen the $G_i(-M)$ values are generally higher than in oxygen-free atmosphere. In a 0.1 M histidine solution, irradiated in the presence of oxygen, the initial decrease of histidine attains the value 6.5. In this medium the $G_i(-M)$ values maintain an increasing tendency within the whole concentration range studied. Evidently, in addition to OH, HO_2, and O_2-radicals a direct participation of oxygen is also operative, under formation of labile peroxy-radicals of histidine of ROO· type [4, 9, 12].

The losses of histidine were compared (Fig. 3) which were observed in neutral 0.02 M histidine solutions irradiated both in the presence or in the absence of oxygen, with those observed in the presence of radical scavengers (N_2O and *sec*-butanol). From Table II it follows that in N_2O saturated atmosphere where a conversion of hydrated electrons to OH radicals takes place the $G_i(-M)$ value of histidine increased by about 40% with respect to the $G_i(-M)$ value observed for oxygen-free medium indicating a distinct participation of the OH

Table I. $G_i(-M)$ values of histidine in variously concentrated histidine solutions, irradiated in the atmosphere of nitrogen and oxygen.

Concentration [M]	0.1	0.05	0.02	0.01	0.005	0.001	0.0005
$G_i(-M)-N_2$	3.5	3.5	3.3	2.9	2.6	2.0	1.9
$G_i(-M)-O_2$	6.5	5.7	5.0	4.5	4.0	3.1	2.7

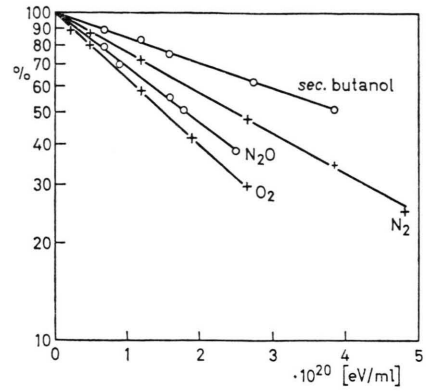

Fig. 3. Losses of histidine irradiated in neutral 0.02 M aqueous solution in the presence of O_2, N_2, N_2O and *sec*-butyl alcohol.

Table II. $G_i(-M)$ values of histidine obtained after the irradiation of neutral 0.02 M solutions of histidine in the presence of radical scavengers and in nitrogen.

Medium	1 M *sec*-butanol	O_2	N_2O	N_2
$G_i(-M)$	2.1	5.0	4.6	3.3

radicals in the radiolysis of histidine. This is also supported by the results obtained on radiolysis of histidine irradiated in a nitrogen washed solution in the presence of *sec*-butanol. The effect of *sec*-butanol as a scavenger of OH radicals leads to a 40% decrease of the radiation loss of histidine.

From the comparison of the $G_i(-M)$ values of the 0.02 M neutral solutions of histidine irradiated in the medium with N_2, N_2O, and N_2 + *sec*-butanol it can be concluded that about 40% of the loss of histidine is caused by OH radicals; the remaining part can be attributed to the effect of e_{aq}^- and H atoms.

The dependence of the radiation decrease of histidine on pH is connected with various ionized forms of this compound (Fig. 4).

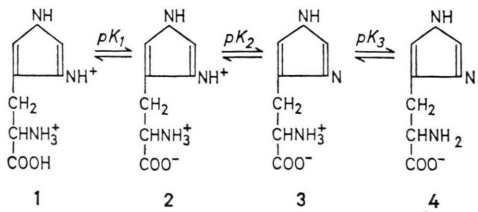

Fig. 4. Ionized forms of histidine (pK_1 = 1.8; pK_2 = 6.0, pK_3 = 9.2).

In the oxygen-free medium the initial radiation decrease of histidine irradiated in acid solution corresponds approximately to the value of about 1.5 (Fig. 5). At low pH predominantly OH radicals

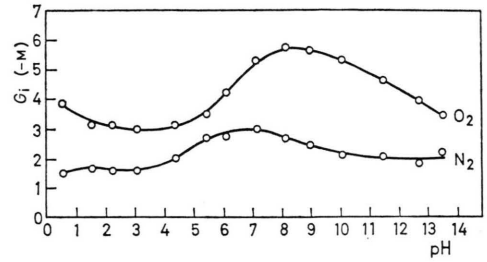

Fig. 5. Effect of pH on the radiation losses of histidine irradiated in 0.02 M aqueous solution under oxygen and nitrogen (pH was adjusted by addition of A grade sulfuric acid and NaOH).

and H atoms react in the irradiated solutions, the proportions of which increase in acid medium in consequence of the protonation of hydrated electrons. According to the results of Draganic [13], hydrated electrons begin to play a role in irradiated solutions from about pH 4.5. They react with histidine molecules which exist in pH 5 – 6 range predominantly in the form a monovalent cation, in pH 7 – 8 range in the form an amphoteric molecule. The pH 5 – 8 region corresponds to that of maximum radiation losses of histidine irradiated in oxygen-free medium, when $G_i(-M)$ corresponds to the value of about 3.2. With increasing pH the reactivity of hydrated electrons with histidine decreases gradually ($k_{pH\,5.9} = 3.9 \times 10^9$ M^{-1} sec^{-1}, $k_{pH\,11.1} = 1.2 \times 10^{-7}$ M^{-1} sec^{-1}), according to the results obtained by Anbar, Bambenek, and Ross [14]. This could explain the decrease of the radiation sensitivity of histidine in alkaline solutions; $G_i(-M)$ value corresponds in this case to the value 2.

During the irradiation of histidine in the presence of oxygen in acidified solutions the radiation loss $G_i(-M)$ attains a value of about 3. In the further course an increase in radiation sensitivity of histidine with increasing pH may be observed. The region of maximum radiation losses of histidine is shifted in the presence of oxygen and forms a broad maximum in the pH 6 – 11 region.

High radiation losses of histidine in this pH region show that ionized forms of histidine, pre-

dominating in this area, are highly reactive to radicals the formation of which is dependent on the presence of oxygen.

The radiation products formed in 0.02 M neutral solutions irradiated in the oxygenated medium were studied. The following substances were identified by means of standards or according to the chromatographic behavior described[15–19]: asparagine, aspartic acid, glutamine, OH-glutamine, glutamic acid, serine, glycine, imidazolyllactic acid, imidazolylacetic acid, imidazolylpyruvic acid, imidazole-4-carboxylic acid, HO-glutamic acid, histamine, imidazole, alanine and α-ketoglutaric acid (the order of enumeration corresponds to the order of their decreasing amount in the irradiated histidine solution). Further radiolytic products of histidine formed, which could not be characterized unambigously, were characterized according to their positive or negative reaction with Pauly's reagent, as compounds containing or lacking an imidazole ring; their relative amount was determined radiometrically.

During irradiation in oxygen-free medium the mentioned products were formed in substantially lower yields (with exception of histamine). In addition to them imidazolylpropionic acid and urocanic acid were detected in irradiated oxygen-free solution of histidine. The recombination products of histidine were the main radiolytic products formed in this medium. During the irradiation with the highest dose about 1% polymer and 27% of radiation products of molecular weight about 330 were detected. The yields of the main radiation products of histidine were summarized in the Table III. From the yields of amide-bound ammonia and from the yields of aspartic and glutamic acids the part of the radiolytic reactions leading to the cleavage of the imidazole nucleus can be approximately estimated, with reference to the total radiolytic decomposition of histidine. In oxygen-saturated solutions irradiated with a 3.17×10^{20} eV/ml dose this fraction is about 73%. The balance of radioactivity of all products including substances the identity of which could not be determined, leads to the similar conclusion: the sum of the radioactivities of the products the imidazole ring of which remained intact, *i. e.* of substances giving a positive Pauly test, is about 30% of the total loss of histidine. The sum of the radioactivity of the products which were formed in consequence of the cleavage of the imidazole nucleus is about 70%. The high yield of free ammonia is in agreement with the reactions mentioned. Hasselmann and Laustriat[20] who studied the photolysis of histidine found the cleavage of the imidazole ring of histidine taking place in the presence of oxygen only. According to them the imidazole ring rupture proceeds through the formation of labile histidine peroxydes to the aspartic acid.

Table III. The radiation losses of histidine and the yields of the main radiation products obtained after irradiation of 0.02 M oxygenated and oxygen-free solutions of histidine on dependence of the radiation doses absorbed. (Expressed in 10^{18} molecules/ml).

Medium	Dose [10^{20} eV/ml]	Decrease of histidine	NH_3 free	NH_3 amide-bound	Aspartic acid	Glutamic acid	Deamination products *	Glycine serine	Yield of histidine molecules in combination products
	0.28	1.45	1.25	0.65	0.06	0.05	0.28	0.3	—
	0.86	3.70	3.00	1.90	0.20	0.17	0.65	0.65	—
O_2	1.43	5.40	4.60	3.25	0.40	0.25	0.85	0.80	—
	3.17	8.80	8.65	5.35	0.70	0.40	1.05	1.1	—
	0.85	2.50	2.10	0.70	traces		0.11	+	0.24
	1.43	3.80	3.35	1.10	0.02	0.015	0.2	+	0.44
	2.55	6.00	5.12	1.70	0.04	0.03	0.32	+	1.32
N_2	3.17	6.95	5.95	1.95	0.05	0.035	0.38	+	1.80
	3.42	7.40	6.45	2.10	0.05	0.04	0.40	+	2.20
	4.35	8.40	8.80	2.20	0.07	0.05	0.47	+	3.40

* Total yields of the products formed during deamination of the α-NH_2 group in the side chain of histidine, i. e. imidazolylpyruvic acid, -acetic acid, -lactic acid and -propionic acid; in oxygen-free solutions the yield of urocanic acid should also be added to this sum.

\+ Qualitative evaluation only.

On radiolysis of histidine however some additional mechanisms of imidazole nucleus cleavage may not be excluded. Comparing the yields of the products formed during the irradiation of histidine with a 3.17×10^{20} eV/ml dose in oxygenated and oxygen-free solutions it becomes evident that the decomposition of the imidazole ring of histidine takes place in the absence of oxygen too but to a restricted extent. The yield of these reactions remains in oxygen-free medium about 30% of the total radiolytic decomposition of histidine. According to the results of Rao, Simic and Hayon [4] obtained during the pulse radiolysis of histidine, the cleavage of imidazole ring of histidine may probably proceed also through the formation of labile OH·-adducts of histidine and through their decomposition to the asparagine and other amides respectively.

Making a balance in oxygen-free medium is more complex because the radioactivity of the recombination products of histidine which also give a positive reaction with Pauly's reagent should be added to the sum of the radioactivity of the products with intact imidazole ring. From the electrophoretic behavior of these substances it follows that the recombination products of histidine of various types are formed – from substances of distinctly basic character, to substances of neutral character. In view of the high yield of free ammonia it may be assumed that in addition to histidine molecules its deamination products, or event the products which are formed by the cleavage of the imidazole ring could take part in the recombination reactions.

From the electrophorograms of histidine solutions irradiated at various pH values and detected with Pauly's and ninhydrin reagents it follows that in acid solutions irradiated under oxygen the proportion of the products formed by the cleavage of the imidazole ring increases in comparison with those obtained from the neutral solutions, *i. e.*, the formation of Pauly's negative and ninhydrin positive products prevails, especially of aspartic and glutamic acid; the maximum of the formation of these products was observed at pH 3. In alkaline solutions the presence of a further radiation product was proved which is of acid nature and does not give a positive reaction either with ninhydrin or Pauly's reagent. It can be assumed that in the presence of oxygen at higher pH the degradation of the imidazole ring takes a different course than in neutral and acid solutions.

When histidine is irradiated in the absence of oxygen the effect of pH on the composition of the radiolysis products again becomes obvious. It was found that the pH of the irradiated solution affects the course of deamination reactions of the side chain of histidine. At pH $0.6 - 1.5$ imidazolylpropionic acid was best represented among the products of deamination, which confirms the decisive effect of H atoms in this region of pH. With increasing pH value the proportion of imidazolylacetic acid increased, while in neutral and alkaline medium imidazolyllactic acid predominates.

From the results presented it follows that the mechanism of the radiolytic degradation of histidine is dependent both on the presence of oxygen and on the ionisation form of histidine, which is dependent on the pH of the irradiated solution.

The radiation transformation of imidazole ring belongs to the main reactions of the free and in simple peptides bound histidine [21] irradiated in aqueous solutions especially in the presence of oxygen. It may be assumed that similar reactions will also take place during the irradiation of longer peptide chains. When the imidazole ring of histidine is in the exposed part of the polypeptide the biological activity of the irradiated substance may thus be substantially altered.

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