Gamma-Radiolysis of Aqueous Solution of Proline

Jiřina Kopoldová and Milan Voráček-Hübsch

Isotope Laboratory of the Institutes for Biological Research, Czechoslovak Academy of Sciences, Prague

(Z. Naturforsch. **30 c**, 474—477 [1975]; received November 15, 1974/February 26, 1975)

Gamma-Radiolysis, Aqueous Solution, Proline, Radiation

Gamma-radiolysis of 0.05 M aqueous neutral proline solution was investigated. As the main reaction of proline radiolysis in oxygenated solutions, the hydroxylation reactions leading to hydroxyproline formation and the reactions leading to the total destruction of the pyrrolidine ring were shown. In oxygen-free solutions the yield of these reactions decreased; in this medium combination reactions giving rise to higher molecular weight combination products (up to approx. 1500) belong to the most important ones. In addition to radiation-decarboxylation reactions the products of radiation-carboxylation reactions were also detected in the irradiated solution of proline free of oxygen.

The study of radiation transformations taking place in irradiated amino acid solutions represents an important contribution to the elucidation of radiolytic changes in irradiated solution of peptides and proteins.

In contrast to other amino acids relatively little attention has been devoted up to now to the radiolysis of proline. Kargaonkar [1] described decomposition of proline and hydroxyproline after gamma-irradiation in aqueous solutions into 7 – 8 products, which, however, have not been identified sufficiently. The formation of hydroxyproline as a consequence of gamma-irradiation of aqueous proline solution was shown by Hurych [2] and by Duzenkova [3]. The photolysis of aqueous solution of proline was studied by Pavolini [4], who demonstrated the cleavage of pyrrolidine ring under the effect of UV irradiation. Radiation scission of pyrrolidine ring was described by Getoff and Schenck [5], who studied radiation carboxylation reactions. In irradiated solution of pyrrolidine they found beside proline some aliphatic amino acids as the products of radiation degradation of pyrrolidine cycle.

According to the rate constants measured for the reactions of proline with e_{aq}^- ($= 10^6 - 10^7\ M^{-1}\ s^{-1}$), OH radicals ($= 1.8 \times 10^8\ M^{-1}\ s^{-1}$) and hydrogen atoms ($= 0.5 \times 10^6\ M^{-1}\ s^{-1}$), proline may be classified among relatively poorly radiosensitive amino acids (Anbar and coworkers [6]). However, in view of the high content of this substance in collagen and elastin that form the main components of the connective tissue we considered it as useful to get some more detailed informations on the radiation transformation of proline as well. Simultaneously we tried to check to what extent the radiation chemical reactions generally described for the radiolysis of aqueous solutions of amino acids (Liebster and Kopoldová [7]) play a role in the radiolysis of this secondary cyclic amino acid.

Requests for reprints should be sent to Dr. J. Kopoldová, Isotope Laboratory of the Institutes for Biological Research, Czechoslovak Academy of Sciences, *Prague*, Czechoslovakia.

Experimental

0.05 M proline (Reanal, Budapest) in aqueous solution (pH 5.6), to which [U-^{14}C]proline (UVVVR, Prague) was added to final radioactive concentration about 10 μCi/ml, was irradiated in glass ampoules. A part of the samples was bubbled through with nitrogen and sealed before irradiation, a part of the samples were bubbled with oxygen during the irradiation. As radiation source ^{60}Co was used, dose rate 1.264×10^6 rad/h (7.73×10^{19} eV/h).

The radiation changes taking place in the irradiated proline solutions were followed by paper chromatography and thin-layer chromatography on silica gel (Silufol) in systems: I. *n*-butanol-acetic acid-water (4:1:5) and II. phenol-ethanol-water (2:1:1). Further paper electrophoresis in pyridine-acetate buffer of pH 5.6 was used at 1200 V, and for the determination of pyrrolidine and hydroxypyrrolidine – in citrate buffer of pH 3.8 (Langemann and Hennegger [8]. In some instances electrophoresis on a Sephadex G 50 (Superfine) layer was used, in 1 M acetic acid at 400 V.

Isatin reagent (Hrabetová and Tupý [9]) and 0.2% ninhydrin solution in aceton, were used for the detection of chromatograms and electrophorograms.

Identification of the separated radiation products was carried out using corresponding standards.

When labelled compounds were used the spots of proline and its radiolytical products separated on chromatograms or electrophorograms were cut out on the basis of corresponding autoradiograms and then evaluated by the scintillation method on a Isocap, Nuclear Chicago, apparatus. Ammonia and volatile bases, including pyrrolidine and hydroxypyrrolidine, were separated by the Conway diffusion method and determined titrimetrically and radiometrically. The recombination products from irradiated solutions were separated on a Sephadex G 10 and G 15 columns (60 cm height, 1.6 cm diameter; elution with 0.1 M acetic acid).

For the study of carboxylation reactions [^{14}C] $NaHCO_3$ was used which was added to the solution of inactive proline before irradiation under nitrogen. Pyrrolidine-[^{14}C]dicarboxylic acid formed in this case was separated during electrophoresis and chromatography on the paper.

Results and Discussion

The decrease in proline content in irradiated solutions is represented in Fig. 1. From the slope of the straight lines, expressing in a semilogarithmic scale the dependence of proline content on the irradiation dose, the initial losses of proline irradiated in the absence of oxygen $G_i(-M) = 1.85$, and in the presence of oxygen $G_i(-M) = 3.3$ were calculated.

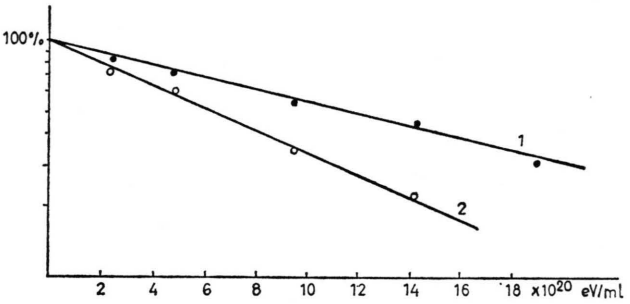


Fig. 1. Loss of proline irradiated under nitrogen (1) and under oxygen (2).

In Table I the G values of proline decrease and of the formation of the main decomposition products are given, which were obtained on irradiation of a 0.05 M solution of proline with a dose 14.22×10^{20} eV/ml (2.3×10^7 rad) under oxygen and nitrogen.

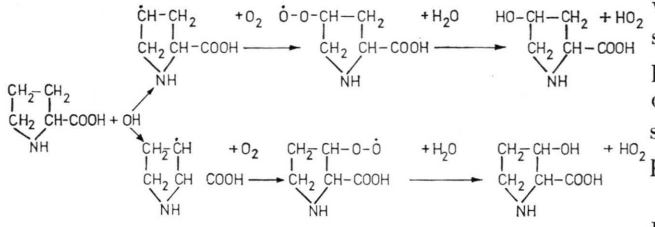
3-OH- and 4-OH-prolines belong to the main products of proline radiolysis in oxygen saturated solution. Their content in comparison with results obtained under nitrogen indicates the direct role of oxygen in hydroxyproline formation probably *via*

Table I. G-values of the decrease of proline and of the formation of some radiation products in 0.05 M proline solution irradiated with a 14.22×10^{20} eV/ml dose in oxygenated and oxygen-free atmosphere, and their chromatographic characteristics.

	G-values		R_F-values		Color reactions	
	N_2	O_2	I.	II.	ninhydrin (on heating)	isatin
proline	−1.15	−1.6	0.31	0.88	yellow	blue
hydroxyprolines	0.05	0.2	0.24	0.6−0.65	orange	blue
β-alanine	0.02	0.1	0.30	0.63	blue-violet	—
glycine	0.02	0.1	0.18	0.43	violet	—
γ-aminobutyric acid	0.01	0.02	0.38	0.76	violet	—
aspartic acid	—	0.04	0.14	0.17	blue-violet	—
δ-aminovaleric acid	0.02	—	0.48	0.81	violet	—
pyrrolidine-dicarboxylic acid	0.02	—	0.25	0.28	pink	blue
glutamic acid	0.01	—	0.25	0.26	violet	—
pyrrolidine	0.02	0.02	0.55 *		yellow	blue
hydroxypyrrolidine	—	0.03	0.43 *		orange	blue
ammonia	0.11	0.2				
yields of proline molecules in combination products:						
1. (m.w. ≈ 1500)	0.23	—	0.00	streak	brown-violet	blue
2. (m.w. ≈ 1100)	0.12	—	0.04	streak	grey-violet	blue
3. (m.w. ≈ 500)	0.15	—	0.14−0.16	0.58−0.68	bluish-green	blue

* Paper previously impregnated with citrate buffer pH 3.8 (Langemann and Honnegger [8]).

organic peroxides of proline:



As follows from Fig. 2, curve 2, the hydroxyprolines formed are simultaneously radiolytically decomposed. In irradiated oxygen-saturated solution of proline a high number of radiolytic products are formed. Part of them was identified as aliphatic

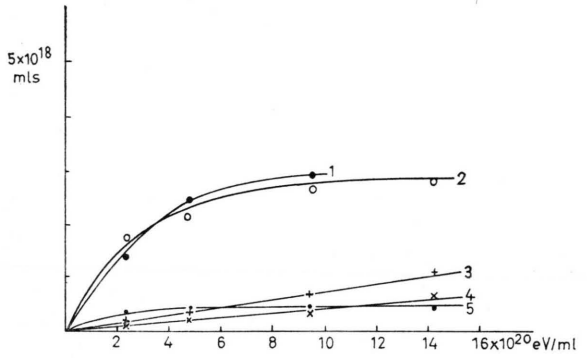


Fig. 2. Formation of radiation products in an irradiated 0.05 M proline solution saturated with oxygen in dependence on irradiation dose. (Expressed in numbers of molecules in one millilitre) 1—volatile bases, 2—hydroxyprolines, 3—β-alanine, 4—glycine, 5—γ-aminobutyric acid.

amino acids: glycine, β-alanine, γ-aminobutyric acid and aspartic acid. Their amounts increased with the dose of irradiation (Fig. 2, curves 3, 4 and 5), and correspond to 16% of $G(-M)$ value of proline, irradiated in oxygenated solution with the dose 14.22×10^{20} eV/ml. Another part of decomposition products didn't give a positive reaction with ninhydrin and was mainly of acid character. Their content determined by radiometric scanning of two-dimensional chromatograms and electrophorograms was expressed in total an corresponds to 8% of $G(-M)$ value of proline. There are products of radiation deamination of proline molecule. In addition to this an appreciable amount of degradation products of volatile character was formed on proline radiolysis. The measurement of the radioactivity of unirradiated and irradiated [^{14}C]proline solutions before and after evaporation indicated total destruction of pyrrolidine ring: The fraction of volatile radiation products amounts to 50% of the total decrease of the original radioactivity of proline when irradiation was carried out in oxygen-saturated solution. The identification of these volatile products was not carried out in this paper. As decarboxylation products formed in irradiated proline solution under oxygen the hydroxypyrrolidine was proved in addition to pyrrolidine.

When the oxygen-free proline solution was irradiated the formation of hydroxyprolines decreased substantially (Fig. 3, curve 2). The scission of pyrrolidine ring is also diminished as indicated by the presence of a small amount of glycine, β-alanine and γ-aminobutyric acid (Fig. 3, curves 3, 4 and 5), and also by decreased amount of volatile and unvolatile degradation products.

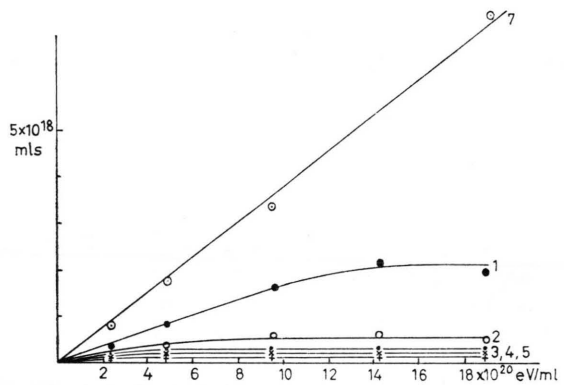


Fig. 3. Radiation products formed in an irradiated 0.05 M proline solution saturated with nitrogen in dependence on irradiation dose. (Expressed in numbers of molecules of radiation products formed in one millilitre; combination products were expressed in numbers of proline molecules in one millilitre, consumed during combination processes.) 1—volatile bases, 2—hydroxyproline, 3—β-alanine, 4—glycine, 5—γ-aminobutyric acid, 7—combination products.

As a product of radiation decarboxylation of proline in oxygen-free solution only pyrrolidine was identified. Simultaneously with decarboxylation the radiation carboxylation reactions also take place in solution irradiated in the absence of oxygen. During the irradiation of inactive proline in the presence of [^{14}C]$NaHCO_3$ the addition of [$^{14}CO_2^-$] to pyrrolidine radical was demonstrated under the formation of [^{14}C]proline, similarly as was described for the other amino acids (Liebster, Kopoldová and Babický [10]). In an irradiated proline solution another radioactive product was also demonstrated which had an acid character, giving with isatin blue and with ninhydrin a pink colour and brick-red fluorescence. It is evidently pyrrolidine-

dicarboxylic acid formed on addition of $[^{14}CO_2^-]$ to proline radical.

Among other products detected in the irradiated oxygen-free proline solution δ-aminovaleric acid was identified, formed evidently by reductive opening of the pyrrolidine ring. A small amount of glutamic acid was also demonstrated in the irradiated proline solution. The mechanism of its formation has not been investigated in this study.

To the main products of proline radiolysis in an oxygen-free atmosphere belong combination products (dimers etc.). Their formation increased with the dose of irradiation (Fig. 3, curve 7). The total amount of combinates formed in proline solution irradiated with a dose 14.22×10^{20} eV/ml corresponds to 39% of $G(-M)$ of proline. These products were isolated from the irradiated solution using Sephadex G 10 column (Fig. 4). As the first fraction (1 and 2) the fluorescing substances were eluted which give with ninhydrin a brown-violet colour, with isatin a light-blue one.

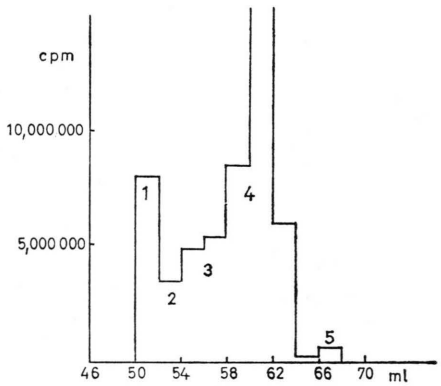


Fig. 4. Separation of 0.05 M solution of [^{14}C]proline irradiated in oxygen-free atmosphere on a Sephadex G 10 column (lenght 60 cm, diameter 1.6 cm, flow rate of 0.1 M acetic acid 10.8 ml per hour). Fractions 1, 2 and 3 correspond to combination products of proline (m.w. cca 1500, 1100 and 500); fraction 4 to proline and some of ith degradation products, fraction 5 to δ-aminovaleric acid. (Expressed in counts of radioactivity.)

Their study by chromatography and electrophoresis on paper and thinlayer was complicated by the appreciable absorption of these substances onto the carrier surface; in system I. the main part remains on the start of chromatograms, in system II. they form streaks in the direction of the development. It was the electrophoresis on a Sephadex G 50 (Superfine) plate which showed that the main combination products are independent substances with distinctly basic character. Evidently combination of proline with the products of its decarboxylation takes place.

The mobility of these combination products isolated from the irradiated proline solution was compared with the mobility of standard samples and oxidized glutathione, bacitracine, oxytocin and polymyxin on a Sephadex G 15 column. From the graphical semilogarithmic representation of the dependence of the mobility on molecular weight of the peptides investigated the molecular weight of the first combination product was estimated to be about 1500, of the second one about 1100.

During the separation on Sephadex G 10 column the presence of lower combination products was also demonstrated in the irradiated proline solution which eluted from the column before proline and other degradation products. They are neutral substances giving with isatin a blue and with ninhydrin a green to green-blue colour (fraction 3). Their molecular weights correspond to about 500. It is not out of the question that these substances are precursors of the main combination products.

Combination reactions taking place during the irradiation of aqueous solution of proline in oxygen-limited atmosphere may play an important role in radiolysis of proline-containing peptides or even in radiolysis of proteins with a high content of proline residues.

[1] K. Kargaonkar, S. V. Marathe, and K. A. Chaubal, Sci. Cult. **34**, 100 [1968].
[2] J. Hurych, Hoppe-Seyler's Z. Physiol. Chem. **348**, 426 [1967].
[3] N. A. Duzenkova and J. Popoldová, DAN USSR, in press.
[4] T. Pavolini, R. Cultrera, and P. G. Pefferi, Gazz. Chim. Ital. **91**, 706 [1961].
[5] N. Getoff and G. O. Schenk, Radiat. Res. **31**, 486 [1967].
[6] M. Anbar, D. Meyerstein, and P. Neta, J. Chem. Soc. **13**, 742 [1966].
[7] J. Liebster and J. Kopoldová, Advan. Radiat. Biol. **1**, 157 [1964].
[8] H. M. Langemann and C. G. Henneger, Anal. Biochem. **8**, 529 [1964].
[9] E. Hrabětová and J. Tupý, J. Chromatogr. **3**, 199 [1960].
[10] J. Liebster, J. Kopoldová, and A. Babický, Radiat. Res. **19**, 551 [1963].