RADIOLYSIS OF AQUEOUS AND AQUEOUS-ETHANOLIC SOLUTIONS OF HISTAMINE

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SUMMARY

Aqueous solutions of histamine and imidazole were gamma-irradiated in the absence and presence of ethanol. The protective effect of ethanol on the radiation cleavage of the imidazole ring depends on the pH of irradiated solutions: at neutral pH the presence of ethanol decreases radiation degradation of imidazole skeleton whereas at acidic pH it does not play any protective role. On the contrary in irradiated aqueous-ethanolic solutions of histamine 2 HCl (pH 4.5) some additional reactions between histamine and products of radiolysis of ethanol, mainly with acetaldehyde, take place leading to the increase of total radiation loss of histamine and to the formation of further radiation products. Similar products were also found in ^{3}H preparations of histamine stored in aqueous-ethanolic solutions. Possible mechanisms of radiation degradation of histamine were discussed.

Key Words: Histamine, Gamma-radiolysis, Aqueous and aqueous--ethanolic solutions, Imidazole ring degradation, Beta-autoradiolysis

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INTRODUCTION

Ethanol is commonly used as a scavenger of OH radicals to suppress autoradiolytical degradation of labeled preparations stored in aqueous solutions. When comparing the preparations of ^{3}H-histamine, which were stored in pure water and in aqueous solutions of ethanol, distinct differences in the composition of the autoradiolytical products in both cases have been observed. We therefore investigated the role of ethanol in the radiation degradation of histamine especially of its imidazole skeleton in greater detail.

For this purpose we used inactive aqueous solutions of histamine 2 HCl at pH 4.5 to which we also added ethanol and ^{14}C-ethanol and then irradiated them with an external source of gamma-radiation. We tried to explain how ethanol acts during radiolysis of histamine and whether it takes a direct part in radiation transformation of histamine.

EXPERIMENTAL

Histamine dihydrochloride puriss. and imidazole (both products of Koch-Light Laboratories Ltd.) were irradiated in pure water and in 2 % or 50 % ethanol (UV sp.Lachema, Brno). The concentration ranged from 5.10^{-4}M to 2.10^{-2}. The solutions were irradiated in glass ampoules with ^{60}Co of 305.4 krad/h intensity. The doses ranged from 0.3 to 5 Mrad. The samples were not deaerated.

In some instances /1-^{14}C/-ethanol (Rotop, GDR) of specific activity 21 mCi/mmol was added to the histamine solutions before irradiation. The preparations of ^{3}H-histamine (prepared in the authors´ institute) of 6-15 Ci/mmol spec. activity were used for the study of autoradiolysis.

The degradation of histamine and the formation of products of radiolysis were estimated by means of electrophoresis on Whatman No.3 paper in pyridine-acetate buffer of pH 5.6 (1400 V). Detection was carried out using Pauly´s diazotization reagent and ninhydrin, or also by radiometric evaluation with linear analyser LB 282.

Quantitative determination of the radiation loss of histamine and of the yields of radiation products was carried out using a Varian gas chromatograph with flame-ionization detection. Glass columns (200 x 2 mm) were used, packed with 3 % OV-17 and 3 % OV-210 silicone stationary phases on Vareport 30 (100-120 mesh) as support. Nitrogen served as carrier gas, its flow rate was 16 ml/min. The columns were heated using a program 150° (3´-10°)min-270° for the OV-17 packing and 134° (1´-10°) min- 290° for the OV 210 packing.

The irradiated histamine samples were converted to volatile derivatives before GC analysis, using heptafluorobutyric anhydride for derivatization[1]. When determining the amino acids formed on irradiation, the irradiated histamine solutions were first esterified and then submitted to acylation[2]. Quantitative analysis was carried out using the method of internal standard (with octadecane) and the quantitative data were obtained with a Varian-CDS 111 calculator.

On the basis of the concentration decrease of histamine versus radiation dose plot, the dose D/37/ i.e. a dose at which the original histamine concentration was decreased to 37 % was determined and the value of the initial radiation loss, $G_i(-M)$ was calculated.

The determination of volatile aldehydes was also carried out by means of GC on columns packed with Separon SDA or Separon BD (of 0.125-0.2 mm particle size) at the programmed temperature 140°/3´-3°/min-170° and 25 ml/min flow rate.

Irradiated solutions of imidazole were evaluated spectrophotometrically at 210 nm.

The determination of the free and amide bound ammonia was carried out by the combined microdiffusion method of Conway[3] and colorimetry with Nessler´s reagent.

RESULTS AND DISCUSSION

The results obtained by analysis of the 2.10^{-3}M and 2.10^{-2}M histamine 2 HCl solutions irradiated with gradually increasing doses of gamma-radiation are presented in Table 1. The presence of ethanol does not decrease the radiation loss of histamine 2 HCl irradiated at pH 4.5. The high yields of ammonia and volatile aldehydes were found both in irradiated aqueous and ethanolic--aqueous solutions of histamine indicating the radiation cleavage of the imidazole skeleton of histamine. However some changes in the ratios between the free and amide bond ammonia yields were found. The cleavage of the imidazole ring thus evidently proceeds according to several reaction mechanisms in dependence on the radiation conditions.

One of these mechanisms could be characterized by the yield of amide bound ammonia. In pure 2.10^{-3}M aqueous solution of histamine the proportion of amide bound ammonia is 55 % of the total radiation loss. In this case the results fully correspond to the results obtained in the irradiation of oxygen-saturated aqueous solutions of histidine[4].

Table 1

Radiation losses of histamine, yields of ammonia and volatile aldehydes versus the radiation dose applied to its solutions (expressed by the number of molecules.10^{16} in 1 ml of the irradiated solution)

a./ in 2.10^{-3}M solutions of histamine.2 HCl

Solvent	Dose Mrad	Loss	Free NH_3	Amide NH_3	Volatile aldehydes
	0.5	48	42	traces	
H_2O	0.8	72	63	30	
	1.2	84	83	46	78
	0.5	54	42	traces	
2 % EtOH	0.8	77	67	26	
	1.2	95	96	32	136
	0.5	55	43	traces	
50 % EtOH	0.8	82	78	24	
	1.2	95	97	38	410

b./ in 2.10^{-2}M solutions of histamine.2 HCl

	3	420	372	110	
H_2O	4	528	486	144	
	5	600	580	168	166
	3	528	522	52	
2 % EtOH	4	648	651	69	
	5	732	782	80	350
	3	480	555	55	
50 % EtOH	4	580	680	85	
	5	696	757	100	870

The volatile aldehydes were evaluated only in solutions irradiated with the highest dose. Increased yields of them in 2 % and 50 % ethanol consist partly from radiation degradation of histamine, partly of ethanol alone.

The presence of ethanol decreases the proportion of the amide bound ammonia to 32 % if histamine was dissolved in 2 % ethanol and to 38 % if it was irradiated in 50 % ethanol.

With increasing concentration of histamine (2.10^{-2}M) higher radiation doses were used (from 2 to 5 Mrads), when consumption of oxygen necessarily takes place. The yield of amide bound ammonia decreases in this case both in aqueous solutions (to 28 %) and in ethanolic solutions (to 11 and 14 %, respectively). From a comparison of these results it follows that the reaction mechanism leading to the cleavage of the imidazole ring of histamine under formation of amide bound ammonia takes place both under participation of oxygen and OH radicals. With increasing concentration of histamine the proportion of free ammonia on the total radiation loss of histamine increases. This yield, expressed by the number of molecules, exceeds in the presence of ethanol the number of molecules of the histamine degraded by radiation. Another mechanism, leading to the cleavage of imidazole ring, must also play a role which seems to be independent both on the content of oxygen and OH radicals. Hydrated electrons e_{aq}^- formed besides OH radicals and H atoms in irradiated water should evidently take part in the total radiation degradation of aqueous solutions of histamine, including the cleavage of its imidazole ring.

For better understanding of the role of imidazole skeleton in radiation degradation of histamine the irradiation of imidazole alone was performed. Reaction rate constants of imidazole with e_{aq}^- and OH radicals are known[5]. Whereas the action of OH radicals with imidazole is nearly independent on pH, the action of e_{aq}^- strongly depends on the

Table 2

The radiation degradation of imidazole skeleton versus pH of irradiated 2.10^{-2}M and 2.10^{-3}M solution of imidazole in water and in 50 % aqueous-ethanolic solution

a.) 2.10^{-2}M solution of imidazole, dose 5 Mrad

Solvent	pH	Radiation degradation (%)
	8.1	22.5
H_2O	4.5	35
	6.9	2.1
50 % EtOH	4.5	32

b.) 2.10^{-3}M solution of imidazole, dose 1 Mrad

	6.1	36.5
H_2O	4.1	41.1
	6.1	4.5
50 % EtOH	4.1	37

protonation state of imidazole (pK_a=7.1) and consequently on the pH of irradiated solutions[6]. We irradiated therefore aqueous solutions of imidazole in the presence and absence of ethanol, partly at their original pH (6.9 and 8.1) partly after their acidification with HCl to pH 4.5 (Table 2).

The degree of radiation degradation of imidazole ring was expressed in each case as the average from the results obtained spectrophotometrically and that calculated according to the yields of free and amide bound ammonia detected in irradiated solutions of imidazole. In original solutions

of imidazole round neutral pH the ethanol really plays a protective role: radiation degradation of imidazole is in the absence of ethanol approximately 10 times higher then in its presence. After acidification of imidazole solution to pH 4.5, no protective effect of ethanol was observed. As can be seen, the protective effect of ethanol on the imidazole ring depends on the pH of irradiated solutions of imidazole.

On the basis of the results obtained with irradiated imidazole and histamine 2 HCl solutions it may be considered that e_{aq}^- are similarly involved. In both cases the protective effect of ethanol at pH 4.5 does not occur. On the contrary, it was found that the radiation decomposition of histamine, characterized by the value of the initial radiation loss $G_i(-M)$ is lower when its aqueous solutions are gamma-irradiated than in its ethanolic-aqueous solution within the whole concentration range of histamine (Fig.1).

The results of electrophoresis and GC showed that in the presence of ethanol the formation of further radiation

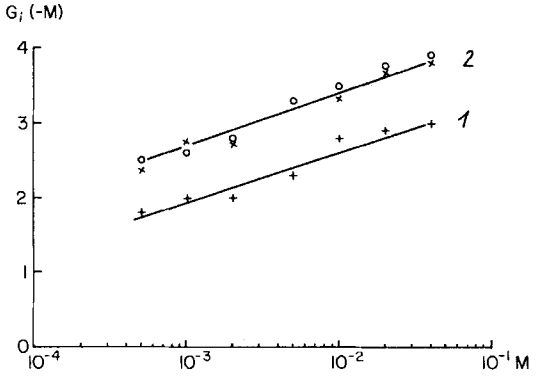

Fig.1 Dependence of the initial radiation loss $G_i(-M)$ on the concentration of the solutions of histamine 2 HCl irradiated in water (1) and in 2 % (o) and 50 % (x) aqueous-ethanolic solutions (2)

Table 3

The results of beta-autoradiolysis of /^{3}H/ histamine

Conditions of storage	Aqueous solution (+4 °C)	50 % Aqueous-ethanolic solution (-20 °C)	
Duration of storage (years)	1.5	3	1.4
Initial specific activity (Ci/mmol)	15.1	5.9	6.0
Radiochemical concentration (mCi/ml)	11.5	15.3	10.5
Initial assumed concentration (M)	$7.5.10^{-4}$	$3.3.10^{-3}$	3.10^{-3}
Calculated absorbed dose (Mrad)	2.6	5.2	1.7
Fraction of volatile radiation products (%)	75	55	25
Content of the histamine (%)	2	9	45
Basic products of autoradiolysis (%)	0	27.5	24.5
Neutral products (%)	17	6.5	5.5
Acid products (%)	2.5	1.5	0

products takes place in addition to the mentioned radiation degradation products of imidazole skeleton. These products were not detected in irradiated aqueous solutions of histamine. There are compounds of basic nature, partly with preserved imidazole structure.

Similar products were found in /^{3}H/ histamine preparations stored in 50 % ethanol at -20 °C whereas in ^{3}H-preparations stored in water at + 4 °C they were not detected (Table 3).

We endeavoured to demonstrate whether ethanol take a direct part in the formation of these radiation products of histamine. Irradiation with a 5 Mrad dose were performed with 2.10^{-2}M solutions of histamine in 2 % and 50 % ethanol, to which /1-^{14}C/-ethanol was added and the radioactivity of irradiated histamine solutions was measured before and after their evaporation to dryness, in comparison with non-irradiated samples. Incorporation of the radioactivity from /1-^{14}C/--ethanol does indeed takes place in the course of irradiation, that is into the group of mentioned basic radiation products of histamine. The total fraction of the incorporated activity is similar as in the case of the irradiation of histamine in 2 % and 50 % ethanol, and it corresponds to about 3.5 % of the original radioactivity of /1-^{14}C/-ethanol. A closer characterization of these products will be subject of our further investigations.

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