Gamma Irradiation of Cytosine in an Aerated Aqueous Solution, II

Kinetic Study of the Formation of the Radiolysis Products of Cytosine Resulting from the Deamination Pathway

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(Z. Nautrforsch. **29 c**, 16–18 [1974]; received July 9, 1973)

Gamma irradiation, cytosine, kinetic

After gamma irradiation of cytosine in aerated aqueous solution, yields of chemical radiation products belonging to the deamination pathway were measured. Primary stable radiolysis products, such as 5,6-dihydroxy-5,6-dihydro-uracil *cis* and *trans* forms, were present with G values lower than those obtained in the radiolysis of uracil irradiated under the same conditions. Cytosine had the same radiosensitivity as uracil ($G = -2.49$).

In preceding work [1], radiolysis products of cytosine resulting from the deamination pathway and biuret have been isolated and identified.

By using radioactive ^{14}C-labelled cytosine and rapid chromatographic techniques, we were able to obtain kinetic curves demonstrating the formation of the principal radiolysis products at doses from 375 rads to several thousand rads.

Results obtained in the present work confirm the earlier hypothesis of Scholes *et al.* [2] and Ekert and Monier [3] who emphasized the role of the 5,6 double bond of the pyrimidine ring in the radiolytic degradation of cytosine in aerated aqueous solutions by gamma rays, and also clearly demonstrated other possible ways of radiation-chemical decomposition.

Results

1. *Irradiation of cytosine with low doses (3000 rad, 10^{-4} M)*

The deamination pathway represented by the uracil radiolysis products (Ducolomb *et al.* [4]) accounts for thirty percent of total amount.

Neither isobarbituric acid nor uracil were identified, and only very low yields of the end-radiolysis products of uracil were measured, such as 5-hydroxyhydantoin, parabanic acid or formylurea.

Not data were available in this low dose range of radiation.

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Table I. G values of radiolysis products of cytosine.

MMM

Products	Uracil 10^{-3} M G Values a	Cytosine 10^{-3} M G Values b	Cytosine 10^{-4} M G Values c
5,6-dihydroxy-5,6-dihydrouracil *cis*	0.42	0.03	0.22
5,6-dihydroxy-5,6-dihydrouracil *trans*	0.62	0.10	0.17
iso-dialuric acid	0.10		0.13
alloxan	0.06		
parabanic acid	0.72	0.03	
5-OH hydantoin	0.18	0.10	0.05
carboxyl ureides	0.13	0.21	0.08
formyl urea	0.27	0.06	0.04
uracil	− 2.48		
$C_4H_7N_3O_4$		0.61	1.28
biuret		0.06	0.01
cytosine		− 2.49	− 2.08

a, Ducolomb *et al*'s data [4]: Total dose: 40 Krads, dose rate: 9 Krads/mn.
b, our data: Total dose: 37.5 Krads, dose rate: 7.5 Krads/mn.
c, our data: Total dose: 3 Krads, dose rate: 3 Krads.

The G values of cytosine disappearance in dilute aqueous solution were similar to those calculated by Scholes [2] (− 2.08 our data − 2.15 Scholes' data). This difference is greater with the value at 10^{-3} M: $G = -2.49$.

This difference can be explained in terms of reactivity of radical species originating from water radiolysis *i. e.* hydroxyl radical ($OH^{\cdot}$) and solvated electron (e^- aq). According to Allen's hypothesis [5], in dilute aqueous solutions radical species are more numerous than substrate one (here cytosine). They

are prevented from reaching the substrate because of rapid recombination.

2. Irradiation of cytosine at 10^{-3} M with high doses of gamma rays (0 to 450 krad).

Initial G values, calculated at 37500 rads (dose rate = 7500 rads/min), where collected in Table I and compared with those obtained by Ducolomb *et al.*[4] under the same experimental conditions.

The G values of cytosine after 37500 rads reaches (−2.49). 5,6-dihydroxy-5,6 dihydro-uracil were produced in relatively poor yields (Table I, Fig. 1).

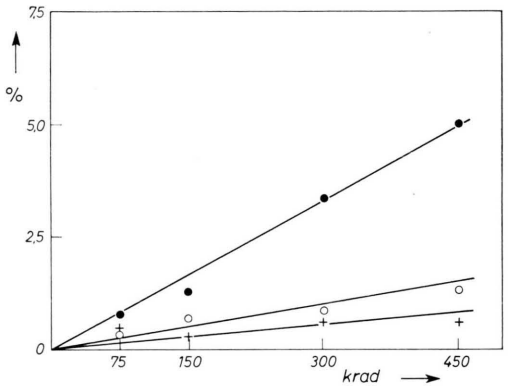

Fig. 1. Formation of 5,6-dihydroxy-5,6-dihydro-uracile *trans* (●), *cis* (○) and biuret (+); (10^{-3} M, dose rate 7.500 rads/mn) from 0 to 450 Krad.

The G value of cytosine was of the same order of magnitude as the values of Scholes[2]. The difference observed could be due to experimental errors but also to the type of technique used.

Cytosine and uracil irradiated under the same experimental conditions had the same radiosensitivity (G cytosine −2.49; G uracil −2.48, Table I).

According to Baxendale[6] the yields of hydrogen atom and hydroxyl radical increase twenty per cent when the pH is lowered from seven to one.

Hence, in an acidic medium (pH 2), radiochemical degradation was accelerated toward end-radiolysis products, such as *N*-glyoxyl-*N'*-formyl urea, 5-hydroxy-hydantoin and parabanic acid. Therefore, it is difficult to distinguish between radiolysis and hydrolysis due to the acidic medium.

The G value of 5-hydroxy-hydantoin equaled that of glycols of uracil. This situation could be explained by the fact that other radiolysis products could also produce 5-hydroxy-hydantoin.

By irradiation of the radiolysis product of cytosine $C_4H_7N_3O_4$, we identified biuret parabanic acid.

By mild alkaline hydrolysis of the irradiated medium (NaOH, N at room temperature overnight),

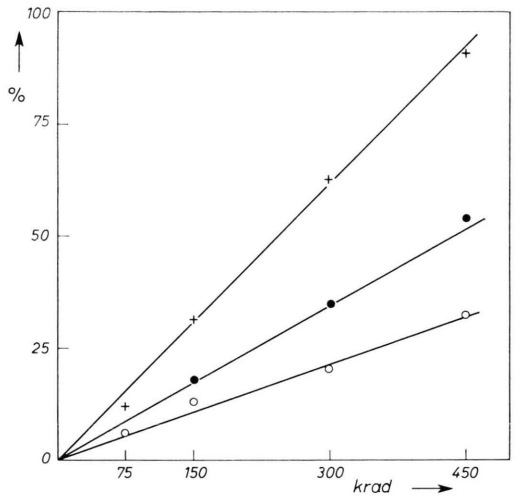

Fig. 2. Comparative percentages of radiolysis products (+) amounts of cytosine degraded, (●) products belonging to the non deamination pathmay, (○) radiolysis products of uracil.

the yield of glyoxylic acid increased with the dose.

In neutral aqueous solutions, products belonging to the deamination pathway, such as uracil glycols, isodialuric acid, etc., were not the most important, but represented nearly thirty per cent of the radiolysis products formed (Fig. 2).

Discussion

Nofre and Cier[8], Khattak and Green[9], and recently Simic and Hayon[10] have emphasized the importance of the ethylenic bond between N_{-3} and C_{-4} as a possible site of radical attack on the pyrimidine ring.

According to Green and Cohen[11], saturated 5,6 dihydro-cytosine compounds lose their amino group very early. The results of Ekert and Monier[3] agree with this mechanism to explain the deamination of cytosine in their own experimental conditions.

A hydroxyl radical attack on the $N_3 - C_4$ bond has been suggested by Khattak and Green[9] in the irradiation of 5-methyl-cytosine in deaerated aqueous solutions. They hypothesized the formation of an unstable intermediate 3-hydro-4-hydroxy-5-methyl-cytosine which lost an NH_3 molecule and gave thymine.

It is difficult to choose between hydroxylic deamination, suggested by Green and Cohen [11], and the second mechanism proposed by Nofre and Cier [8]. But even if we consider an additive effect of these two mechanisms, deamination cannot be taken as the most important radiation-chemical degradation pathway of cytosine.

Experimental Methods

Purification of ^{14}C-labelled cytosine

Commercial labelled [2-^{14}C]cytosine (CEA Saclay France) was purified just before irradiation by two-dimensional thin-layer chromatography (solvents systems A and B, silicagel Macherey Nagel [1]).

Thanks to its fluorescence under ultraviolet light at 254 nm, the radioactive spot of cytosine was scraped off and eluted three times with methanol (analytical grade). The silicagel was roughly eliminated by centrifugation, and then completely removed by filtration of the alcoholic solution through a Millipore filter (GSWP 01300 – 0.22 μ pore size).

The methanol was evaporated under vacuum and the residue washed twice with tridistilled water to avoid traces of alcohol.

Irradiation

The radioactive tracer was mixed with an inert cytosine solution (Fluka). For each dose of gamma ray irradiation, one milliliter was poured into a pre-irradiated glass vial. With constant air bubbling, this solution was irradiated without delay.

The irradiated solution was then evaporated at room temperature. The dosimetry for the gamma rays was made according to the Fricke's technique.

Chromatographic methods

The irradiated residue was taken up with methanol (0.1 ml) and subjected to two-dimensional thin-layer chromatography as in the preceding work [1]. (Silicagel MN-N-HR/UV A and B solvents systems.)

The radiolysis products were detected by autoradiography with a Kodak emulsion (Kodirex type).

The silicagel from the radioactive zones was carefully scraped off the chromatographic plate and poured directly into the counting vials.

Radioactivity measurements

The radiolysis product of each spot was extracted overnight with two milliliters of distilled water, with yield ranging from 90 to 100 per cent.

After this extraction, the counting vials were filled with a dioxan scintillation mixture consisting of 1 liter of dioxan, 6 g of PPO, 0.3 g of POPOP, 100 ml of ethylene glycol, 100 g of naphthalene. Each vial was shaken on a vortex mixer three minutes and finally counted with a Tricarb Model (Packard Instruments).

We wish to thank Mr. Guy, Gauci and Guerin for their helpful technical assistance.

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