THE STUDY OF GAMMA-RADIOLYSIS AND BETA-AUTORADIOLYSIS OF AQUEOUS SOLUTIONS OF THYMIDINE AND URIDINE

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SUMMARY

The effect of external gamma- and internal beta-irradiation on the aqueous solutions of unlabelled and ^3H labelled preparations of thymidine and uridine was investigated within the concentration range 10^{-4} - 2.10^{-2}M. From these investigations corresponding D/50 and $G_i(-M)$ values were calculated and compared. The differences between the $G_i(-M)$ values of labelled and unlabelled preparations obtained in very dilute solutions (up to 5.10^3 - 10^{-2}M) were discussed.

INTRODUCTION

In the preceding paper (1) we investigated the radiolysis of tritiated pyrimidine nucleosides under various conditions of their storage in dependence on the specific radioactivity of the preparations under investigation. The results obtained were utilized in this study for the calculation of kinetic values of the autoradiolytic processes taking place in aqueous solutions of thymidine-6-T and uridine-5-T in consequence of autoirradiation with beta particles during storage. Simultaneously the changes were also investigated which take place in aqueous solutions of active and inactive preparations of these nucleosides under the effect of external gamma-radiation and kinetic values of these radiolytic processes were computed.

We were interested to see whether the radiation processes studied are comparable, or else, whether it is possible to draw conclusions from the results of external radiolysis of inactive substances on the course of the autoradiolysis of their tritiated preparations. Three series of experiments were evaluated in which the following factors were followed:

1) The effect of external gamma-radiation on aqueous solutions of thymidine and uridine,

2) the effect of external gamma-radiation on aqueous solutions of tritiated preparations of these substances,

3) the effect of internal beta-radiation on aqueous solutions of tritiated preparations during their storage.

Experimental part

For the computation of rate constants of autoradiolysis and the initial $G_i(-M)$ values (i.e. the number of decomposed molecules of the irradiated substances per 100 eV of the absorbed energy at dose→0) of thymidine-6-T and uridine-5-T stored in aqueous solutions at $+2^{o}C$ the results presented in the preceding paper (1) were utilized. The original specific radioactivity of thymidine (25 Ci/mmol) and uridine (10 Ci/mmol) was gradually decreased by the addition of the corresponding inactive carrier until it dropped to 1 or 0.5 Ci/mmol. The molar concentration of the studied solutions increased thus from the initial 4.10^{-4} to $10^{-2}M$ in the case of thymidine, and from 10^{-3} to $2.10^{-2}M$ in the case of uridine. The radioactive concentration of the studied solutions remained in all instances constant, i.e. 10 mCi/ml. From this the energy doses of tritium beta-radiation were calculated, which were absorbed in the stored solutions at various stages of their storage.

As source of external gamma-radiation 5 kCi ^{60}Co of 10^5**rad/h** intensity were used, i.e. $6.3 \cdot 10^{18}$eV/**h.** The solutions of inactive thymidine and uridine (Koch-Light Laboratories Ltd.) were irradiated in sealed glass ampoules with increasing doses of gamma radiation at room temperature. Under the same conditions freshly supplied aqueous solutions of tritiated preparations of thymidine and uridine (Institute for the Research, Development and Production of Radioisotopes - ÚVVVR, Prague) were also gamma-irradiated, which were adjusted by the addition of an inactive carrier from the original 4.10^{-5}M concentration up to the 2.10^{-2}M concentration. Neither the stored solutions of tritiated preparations, nor the solutions prepared for gamma-irradiation were specially deaerated.

The content of thymidine and uridine (S) in irradiated samples was followed spectrophotometrically; in radioactive preparations also by radiometric measurement of chromatograms of irradiated solutions. In this case the measured values had to be corrected for the content of volatile radioactive products which were formed in consequence of radiolysis, especially for the content of tritiated water. Their part was calculated from the difference of radioactivity of the evaporated and non-evaporated irradiated solution. The drop in the concentration of thymidine and uridine which occurs in consequence of a direct internal effect T → He (which according to Evans (2) amounts in the case of substances with a higher specific activity up to 5% of the original activity per year of storage) may be neglected in this case.

Results and discussion

The dependence of the radiation degradation of thymidine and uridine (S/S_0) on the irradiation dose, plotted in a semilogarithmic scale, gives a set of curves whose slopes characterize the rate of radiation decomposition at various concentrations of the irradiated solutions. D/50, i.e. the radiation doses at which a 50% degradation of the original substances took place were subtracted from the graphs. From these values the rate constants and $G_i(-M)$ values were calculated for single concentrations of the irradiated nucleosides. The values obtained during the gamma-radiolysis of inactive thymidine and uridine samples are presented in Table 1, column A; the values obtained on gamma-radiolysis of tritiated preparations of these substances are given in the same table in column B.

A similar treatment was also applied to the results obtained during the study of the radiolysis of tritiated preparations stored at various specific activities in aqueous solutions at +2°C. From a drop of the original radioactivity of the stored samples and the values obtained after correction for the content of volatile radiation products the S/S_0 values were determined in dependence on the time of storage, i.e. on the amount of the absorbed energy of the tritium beta-irradiation. In Table 2 the corresponding D/50 values are listed as well as the $G_i(-M)$ values of the beta-radiolysis of tritiated preparations of various specific activity. Initial radiation yields of labelled molecules of thymidine and uridine, $G_i(-M^*)$, are given in this table independently. For their calculation the concentration of tritiated preparations of theoretic specific activity 29.1 Ci/mmol was taken as initial as at this concentration all the molecules of these preparations should have been labelled with tritium, i.e. the 3.2 . 10^{-4}M concentration.

Table 1
Radiolytic Data Concerning gama-Radiolysis of Thymidine and Uridine (A) and their Tritiated Derivatives (B) in Aqueous Non-Deaerated Solutions.

	S_o mol/l $(.10^{-2})$	A		B	
		D/50 eV/ml $(.10^{20})$	$G_i(-M)$	D/50 eV/ml $(.10^{20})$	$G_i(-M)$
Thd	0.04	-	-	0.155	1.08
	0.1	0.155	2.7	0.235	1.78
	0.2	0.29	2.85	-	-
	0.25	-	-	0.410	2.54
	0.5	0.72	2.9	0.7	2.98
	0.8	1.1	3.0	-	-
	1.0	1.34	3.1	1.25	3.3
	2.0	-	-	2.45	3.4
Urd	0.01	0.015	2.8	0.32	0.13
	0.04	0.056	2.95	0.36	0.46
	0.1	0.135	2.9	0.43	0.96
	0.2	0.31	2.85	-	-
	0.25	0.33	3.1	0.55	1.9
	0.5	0.63	3.3	0.75	2.8
	1.0	1.3	3.2	1.3	3.2
	2.0	2.4	3.35	2.5	3.3
	2.5	3.0	3.45	3.1	3.4

In view of the fact that the substances investigated had a relatively low electron density and that the concentrations of the solutions used were sufficiently low, it may be supposed that the main part of the degradation of the investigated substances should take place in both cases, i.e. both on gamma-radiolysis and on beta-autoradiolysis, in consequence of the indirect effect of the radiation, i.e. through the mediation of the radicals and active particles of water. In this case the $G_i(-M)$ values of the irradiated substances, should be independent of the concentration of the irradiated solutions. Only the results from the first series of the observed phenomena agree to a certain extent with this condition, i.e. the results obtained from gamma-radiolysis of inactive preparations of thymidine and uridine. As follows from table 1/A the $G_i(-M)$ values of gamma-irradiated inactive preparations of thymidine and uridine (within the limits of the concentrations investigated) oscillate according to expectations about a constant value ≈ 3, which may be considered as a characteristic symptom of the indirect effect of the irradiation. With the in-

Table 2

Radiolytic Data Concerning beta-Autoradiolysis of Tritiated Thymidine and Uridine in Aqueous Non-Deaerated Solutions Stored at 2°C

Specific activity Ci/mmol		S_o mol/l $(.10^{-2})$	D/50 eV/ml $(.10^{20})$	$G_i(-M^*)$ of labelled molecules only	$G_i(-M)$ of labelled and unlabelled molecules
Thd	25	0.04	0.215	0.57	0.75
	20	0.05	0.265	0.47	0.78
	10	0.1	0.35	0.36	1.2
	5	0.2	0.45	0.28	1.85
	1	1.0	0.96	0.13	4.3
Urd	10	0.1	0.28	0.44	1.5
	5	0.2	0.46	0.28	1.9
	1	1.0	0.88	0.14	4.6
	0.5	2.0	1.9	0.06	4.4

crease of the concentration of the irradiated solutions only a mild increase of this value takes place in this case. Loman and Block (3) found a similar course during the irradiation of aqueous thymine solutions. A rising tendency of $G_i(-M)$ values with increasing concentration of the irradiated solution is explained by the fact that in dilute solution the OH radicals (G_{OH}= 2.7) are mainly responsible for the degradation of the irradiated substance; with increasing concentration of the irradiated solutions other active water particles also take part during the radiation degradation, which leads to a mild increase of $G_i(-M)$ of the dissolved substances. In our case the maximum value of $G_i(-M)$ was 3.4.

In comparison with inactive samples of thymidine and uridine whose $G_i(-M)$ values are almost independent of the concentration of the irradiated solutions, the situation in the irradiated solutions of tritiated preparations of these substances is different.

During the gamma-radiation of very dilute solutions of tritiated nucleosides their $G_i(-M)$ values are appreciably dependent on the concentration of the irradiated solutions. When comparing the results of the gamma-radiolysis of inactive (Tab.1/A) and active (Tab.1/B) preparations, it can be seen that in very dilute solutions the radiation yields of tritiated samples are substantially lower than $G_i(-M)$ values of inactive preparations. This is especially striking in the case of uridine, where active and inactive preparations were irradiated in solution of very low concentrations. From the results it follows that the initial radiation yields of tritiated preparations increase distinctly with increasing concentration of the irradiated solutions. The increase takes place up to the concentration 5.10^{-3}M when a certain stabilisation of $G_i(-M)$ values at about the value 3 occurs and thus approaches the results obtained on gamma-radiolysis of inactive samples. This value then remains constant and does not change even on further increase in concentration of the irradiated solutions.

A similar trend was also observed during the autoradiolysis study of stored preparations of thymidine-6-T and uridine-5-T. It is evident from Table 2 that on autoradiolysis of tritiated samples an appreciable dependence of the initial radiation yield on the concentration of the stored substances is observed, or also on the specific activity at which they are stored. A gradual decrease of the specific activity of tritiated preparations by a homogeneous dilution with the corresponding inactive carrier leads to a decreased degradation of active nucleoside molecules to the detriment of inactive molecules of the added carrier. The initial yield of decomposed labelled molecules of thymidine and uridine decreases on increasing the total concentration, while the initial loss of inactive molecules of these substances distinctly increases with increasing concentration of the solutions studied. A certain stabilisation of the $G_i(-M)$ values of the stored tritiated substances - about the value 4.5 - takes place at a concentration of about 10^{-2}M.

The results obtained in all three series of experiments are summarized in Figure 1, representing the dependence of $G_i(-M)$ values on the concentration of irradiated solutions. When comparing the course of this dependence in the case of active and inactive thymidine and uridine preparations, the difference is immediately evident. Active samples irradiated in dilute solutions either with

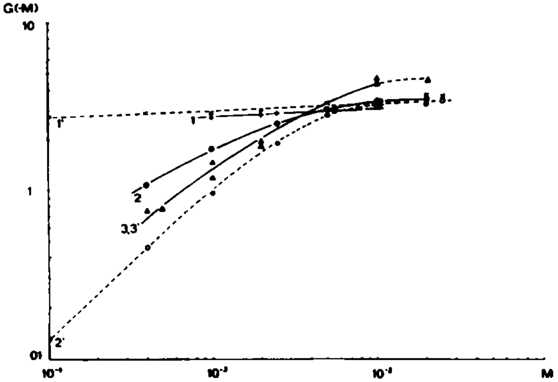
Figure 1

The $G_i(-M)$ values of unlabelled and ^{3}H-labelled thymidine and uridine irradiated in aqueous solutions with gamma-external (1,1′,2,2′) and beta-internal (3,3′) radiation source versus concentration:

1(1′)-gamma-radiolysis of unlabelled preparations of thymidine (uridine)

2(2′)-gamma-radiolysis of ^{3}H-labelled thymidine (uridine)

3(3′)-beta-autoradiolysis of ^{3}H-labelled thymidine (uridine)



external gamma-radiation (curves 2,2′) or with internal beta-radiation (3,3′) are less sensitive toward irradiation than inactive preparations of these substances, when irradiated under the same conditions (1,1′).

This difference evidently follows from the chemical nature of the irradiated samples, i.e. from different procedures used during the isolation and purification of active preparations, which affects the purity of the supplied solutions (chromatographic purification of active preparations on paper, elution of the corresponding zones, etc.). A higher radiation sensitivity of inactive preparations may be decreased by adding to its solution before irradiation a solution of tritiated sample. The higher was the amount of the tritiated sample added, the more the sensitivity of the inactive thymidine sample decrease, i.e. the value of its radiation loss $G_i(-M)$ decreased. The same effect was achieved if a solution of inactive thymidine prepared for irradiation, was additioned instead of tritiated preparation only with the eluate of a pure chromatographic paper through which the solvent system, used for the purification of active substances, had been run.

Hence, the radiation behaviour of tritiated preparations of thymidine and uridine is evidently affected by the presence of admixtures, originating from the previous workup of these preparations. These admixtures act as scavengers of free radicals of water, they protect the molecules of the investigated substances, and decrease the radiation $G_i(-M)$ values.

Similar results were obtained if aqueous solutions of tritiated preparations of thymidine and uridine produced by other firms were externally gamma-irradiated. It was found that even these tritiated preparations are substantially more radiation recistent that inactive samples of these substances irradiated under the same conditions.

In view of various methods used for the isolation and purification of tritiated nucleosides during their preparation, the amount and the quality of the admixtures can be somewhat different as well as their effect on the radiolytic degradation of the investigated substances. This may explain the differing results obtained sometimes in the determination of $G_i(-M)$ of the autoradiolysis of the stored tritiated preparations in various laboratories, or also in various batches.

From the obtained results it follows, that the radiolysis of tritiated nucleosides irradiated in dilute solutions either by external or internal beta-radiation is distinctly affected by the presence of admixtures originating from the previous treatment of tritiated preparations. With increasing concentration of nucleosides the scavenging function of admixtures decreases. During gamma-radiolysis of tritiated preparations the effect of admixtures is eliminated in our case at a nucleoside concentration of about 5.10^{-3}M. From this concentration the value of the initial radiation yields $G_i(-M)$ in irradiated solutions of tritiated nucleosides remains constant (≈ 3), and the course of gamma-radiolysis of active and inactive preparations is practically equal. During beta-autoradiolysis stabilization of $G_i(-M)$ values takes place at a concentration of stored solutions of tritiated nucleosides of about 10^{-2}M corresponding to the specific activity of 1 Ci/mmol. The initial yield of autoradiolytic decomposition of $G_i(-M)$ attains in this case the value of about 4.5. A higher result - in comparison with a gamma-radiolytic decomposition - is due evidently to the higher ionization density of the beta-radiation of tritium.

The mentioned circumstances must be borne in mind if we wish to draw conclusions from the results of external radiolysis of inactive samples on the radiation stability of their tritiated preparations. Only from the results of radiolysis of aqueous solutions of active and inactive nucleosides, beginning at a 5.10^{-3} -

10^{-2}M concentration, certain conclusions may be drawn and the obtained results compared. In very dilute samples the course of radiolysis in these two cases is different.

References

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