STUDY ON AUTORADIOLYSIS OF TRITIATED NUCLEOSIDES

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SUMMARY

The autoradiolytic decomposition of thymidine-6-T and uridine-5-T in aqueous solution at +2 and -15°C, in ethanol, and on chromatographic paper was studied in dependence on time of storage and specific activity.

From practical experience obtained in various laboratories during the storage of tritiated preparations [1-11] it was deduced that the rate of autoradiolysis of these substances is dependent on various factors, such as specific activity of the labelled compounds, time of storage, form in which they are stored, temperature of storage, purity of the preparations, and the presence of substances functioning as scavengers of radicals formed in labelled preparations in the course of their storage (for example ethanol).

In this part we followed the radiation decomposition of tritiated thymidine and uridine in dependence on time of their storage under various conditions, and of various specific activities.

EXPERIMENTAL

The substances investigated were obtained from the Prague Institute for Production, Use and Application of Radioisotopes in the form of aqueous solutions of radiolytic concentration 10 mCi/ml. The specific activity of thymidine-6-T was 25 Ci/mmole, that of uridine 10 Ci/mmole. The declared radiochemical purity of the samples was 98 %; at the time of the beginning of the experiment it was 93 % in the first case and 87 % in the second.

The obtained samples of the tritiated preparations were mixed with calculated amounts of inactive nucleosides in order to decrease their specific activity gradually; from 25 Ci/mmole to 20,10,5 and 1 Ci/mmole in the case of thymidine, and from 10 Ci/mmole to 5,1, and 0.5 Ci/mmole in the case of uridine. In some instances a substance of similar type was added to uridine as an inactive carrier, as for example thymidine, or else a substance of a completely different nature was added, as for example glucose and glycine.

The solutions of thymidine and uridine of various specific activities were pipetted into glass ampules (0.2 ml of the solution) which were sealed at ordinary pressure and sterilized in an oven at 120°C for 20 minutes.

1) A part of the samples was stored at +2°C.
2) Another part was frozen by immersion in a mixture of ethanol and dry ice and then stored at -15°C.
3) Some samples were diluted with an appropriate amount of ethanol to a 3% concentration and then stored at +2°C.

4) The samples were applied under strictly constant conditions onto chromatographic paper, Whatman N°3, i.e. keeping constant the volume of the sample, the method of application and the conditions of drying. The paper strips with the applied samples were stored in evacuated sealed ampullas at +2 and -15°C.

The radioactive concentration of all these samples remained the original one, i.e. 10 mCi/mL. In one instance only a solution of tritiated thymidine of 25 Ci/mmole specific activity was used, which was diluted to radioactive concentration of 0.5 mCi/ml.

In intervals of 3,6,9,12,16, and in some instances 19 months samples were taken from the ampullas stored in various ways, and the latter were sealed again and stored under the same conditions.

The total radioactivity of the stored samples was measured on Mark scintillation counter. The content of volatile decomposition products was determined from the difference of the activities of the original sample and the sample evaporated after the addition of 20 µg of an inactive carrier. The decrease of the original material and the formation of degradation products was followed by paper and thin-layer chromatography and autoradiography in systems: n-butanol--acetic acid-water (4:1:5), n-butanol-water (86:14), and ethyl acetate saturated with phosphate buffer of pH 6.

The chromatograms cut to 1x3 cm strips were measured also on the scintillation counter. (In this case best results were obtained when the samples were placed obliquely into the measuring vials at the bottom of which a few drops of the scintillation solution were added, sufficient for the moistening of the paper strips.)

RESULTS AND DISCUSSION

During the autoradiolysis of thymidine and uridine, degradation of the original substance takes place, accompanied by the formation of volatile and non-volatile decomposition products. The main component of the volatile decomposition products was tritiated water . The mechanism of formation of tritiated water and of other autoradiolytical products just studied in our laboratory will be discussed in the text part of our paper in more detail . In this part the losses of thymidine and uridine of various specific activities stored in various conditions were investigated. Results obtained were expressed in percent of starting radioactivity and plotted in graphs. The time of storage also expresses the energy dose absorbed by the sample of a constant radioactive concentration 10 mCi/ml.

Figures 1 and 2 represent the degradation of thymidine and uridine of various specific activities in dependence on the time of storage at $+2^{\circ}C$. From these graphs it follows that the radiation decomposition of tritiated nucleosides is dependent on the specific activity of the samples investigated. This dependence is represented in Fig.3 where the results of the analysis of thymidine and uridine after one year of storage are given. The decrease of the investigated substances is expressed by the relation S'/S° where S is the observed and $S^{\bullet}$ the starting concentration of their solutions; the

specific activity may be expressed as the molarity of the stored solutions. As follows from the graphical representation the specific activity of samples stored in 3% ethanol

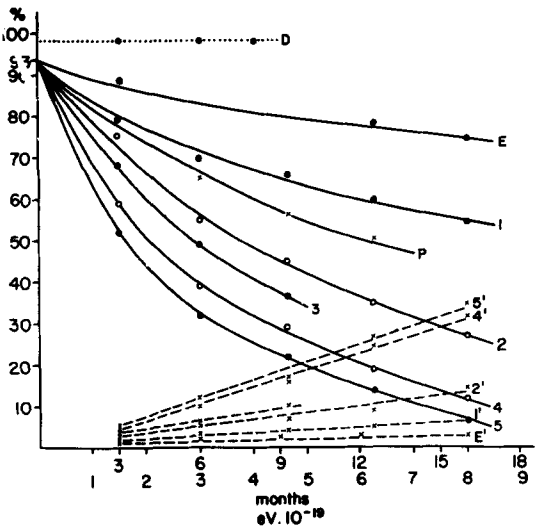

Fig.1: Autoradiolysis of thymidine-6-T stored at +2°C in aqueous solutions with specific activities 1,5,10,20 and 25 Ci/mmol (full lines 1-5), in ethanol (E) and on chromatographic paper (P) in dependence on time of storage resp. on the energy doses absorbed by samples of radioactive concentration 10 mCi/ml. The shaded lines 1′- 5′characterize the formation of tritiated water in these samples. The dotted line corresponds to the thymidine with dilute radioactive concentration 0.5 mCi/ml.

and on chromatographic paper does not affect their radiation decomposition. In contrast to this, in the case of aqueous thymidine and uridine solutions stored at +2°C, the decrease of their specific activity, i.e. the increase of the molarity of the solutions investigated, leads to the decrease of their radiation decomposition.

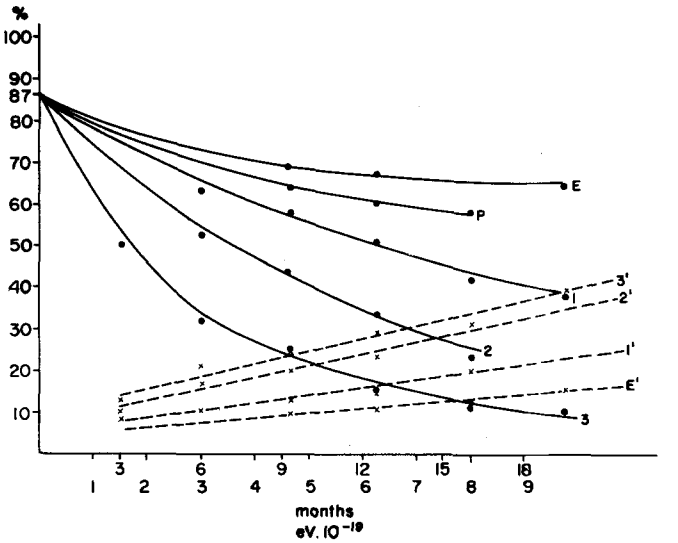

Fig.2: Autoradiolysis of uridine-5-T stored at +2°C in aqueous solutions with specific activities 1,5, and 10 Ci/mmol (full lines 1-3), in ethanol (E) and on the paper (P) in dependence on the time of storage. The shaded lines 1′- 3′correspond to the formation of tritiated water in these samples.

The decomposition of thymidine and uridine stored at -15°C has a similar course with the difference that the samples undergo a stronger decomposition in frozen state than samples stored at +2°C which is in agreement with results obtained and explained by Apelgot [12-15].

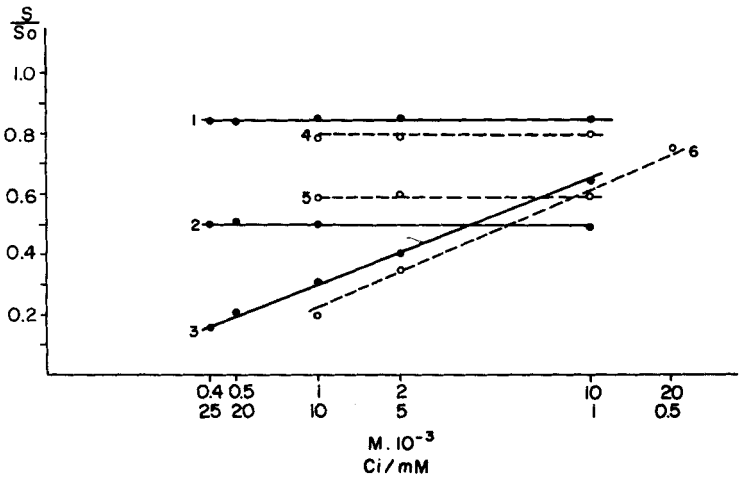

Fig.3: Autoradiolysis of thymidine (full lines) and uridine (shaded lines) after 1-year storage in dependence on their specific activities (25-1 Ci/mmol) expressed as the molar concentrations of these solutions (0.0004-0.05 M) stored in ethanol (1,4), on the paper (2,5) and in water at +2°C (3,6).

In order to increase the molarity of the solutions of tritiated preparations other non-active compounds can also be used which then can be eliminated from the stored solutions, for example by chromatography. Fig.4 shows results obtained after 9 months storing of uridine. The first column indicates the decomposition of uridine of specific activity 5,1 and 0.5 Ci/mmole. Columns 2,3, and 4 represent the decomposition of uridine in solutions to which a corresponding amount of inactive thymidine, glucose and glycine was added. The ad-

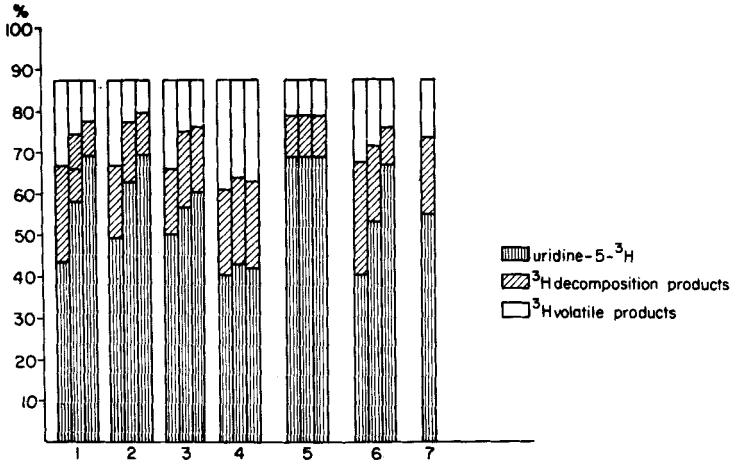

Fig.4: Selfdecomposition of uridine after 9 months storage in aqueous solutions with decreasing specific activity 5,1 and 0.5 Ci/mmol by addition of inactive uridine (column 1), thymidine (2), glucose (3) and glycine (4); in ethanol (5); in frozen state (6) and on the chromatographic paper (7).

dition of inactive thymidine and glucose had the similar effect on the course of radiolytic degradation as the corresponding decrease of its specific activity. The addition of glycine did not lead to a similar effect. Evidently, glycine is more stable from the radiation point of view, which results in the radiation changes taking place preferably in the uridine molecules.

From the above results the following conslusions can be drawn:

The degree of the self-decomposition of the substances investigated depends on the conditions of their storage. The lowest decomposition takes place in ethanolic solutions, where the content of thymidine and uridine of highest specific activities during a one-year storage is diminished by 13-14 %. A stronger decomposition takes place in samples stored on chromatographic paper ; after one year the thymidine content dropped by 40% and that of uridine by approximately 30%. This unexpectedly strong decomposition of tritiated nucleosides stored on paper may be tentatively explained by the non-homogeneous distribution of the sample after their application on paper and immediate storage [16]. During the application and mainly during the drying of the samples the tritiated substance is concentrated in narrow zones on the paper surface. The development of the papers with the applied samples in some of the chromatographic solvent systems and their free drying in air may increase the homogeneity of the sample and thus also their stability.

A deep decomposition takes place in tritiated nucleosides

stored in aqueous solutions at +2°C and even deeper at -15°C. In this case the decrease of the specific activity of thymidine and uridine, or the increase of the molar concentration of these solutions by the addition of other inactive substances leads to a substantial decrease of the percentage of the decomposition of tritiated preparations, and also to the increase of their stability. In the case of one year's storage at +2°C of an aqueous thymidine solution of specific activity 25 Ci/mmole, its content drops to 14%; the content of thymidine of specific activity 1 Ci/mmole, stored under the same conditions, dropped - after the same period - to 60 % of its original concentration.

However, it should be taken into account that the inactive carrier added to radioactive solutions will also undergo radiation degradation. If expressing the decrease of the studied samples of thymidine and uridine as radiation decrease G(-M), i.e. as the number of molecules changing per each 100 eV of the absorbed irradiation dose, we come to results shown in Fig.5. This figure expresses the values G(-M) of thymidine in dependence on the specific activity of the samples, the time and the mode of their storage. It follows that in the case of nucleoside samples stored at lower specific activity the absolute number of changed molecules per 100 eV of the absorbed dose is higher. When specific activity is decreased, i.e. the molar concentration of the stored samples is increased, the total amount of degraded molecules of the inactive compound added also increases, leading to an increase in the concentration of inactive degradation products in the stored preparations.

The lowest degradation of tritiated nucleosides was observed in our study in the case of a thymidine sample whose radioactive concentration was diminished from the original 10 mCi/ml to 0.5 mCi/ml, while the original specific activity 25 Ci/mmole was preserved. It was found that gradual dilution of aqueous solutions of tritiated preparations can suppress the radiolysis of these substances and that their higher stability can be attained under simultaneous preservation of high specific activity, very desirable in the case of these preparations. A more detailed study of the effect

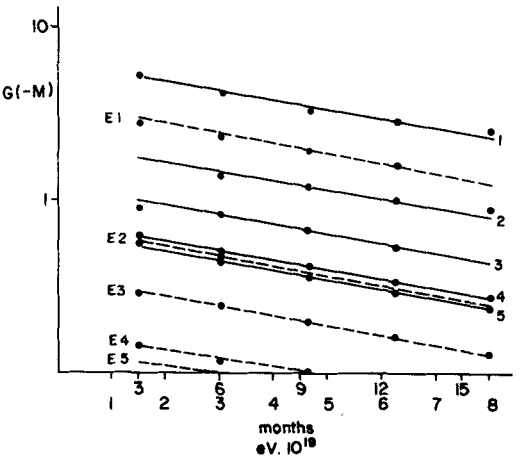

Fig.5: G(-M(values of thymidine of specific activities 1,5,10,20 and 25 Ci/mmol stored in aqueous solutions (full lines 1-5) and in ethanolic solutions (shaded lines E 1 - E 5) at +2°C in dependence on the time of storage.

of the radioactive concentration of tritiated nucleosides on the stability of these substances is the object of our further investigations.

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