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Abstract

Full Text

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THE ACTION OF γ-RADIATION ON GLUCOSAMINE

Radiolysis of carbohydrates and carbohydrate-containing biopolymers is of substantial interest for a correct understanding of the ways in which penetrating radiation acts on biological systems. Although a considerable number of works have been devoted to the action of radiation on the simplest neutral monosaccharides (1), the action of radiation on amino sugars has not been studied. Meanwhile, considering the responsible place that amino sugars occupy as components of the most important biopolymers, such data may be of substantial importance. In the present article we present preliminary data on the study of the action of γ-radiation on glucosamine, chosen as the first example of a biologically important amino sugar.

The source of γ-radiation was Co^60 (40,000 g-equiv.). Aqueous solutions of glucosamine base, concentration $5 \cdot 10^{-2}$ M, were studied. Irradiation was carried out in sealed glass ampoules of 40 ml volume in an atmosphere of nitrogen free of oxygen. The dose rate was $5 \cdot 10^{16}$ eV/ml $\cdot$ sec. The total dose interval investigated was from $0.3 \cdot 10^{19}$ to $18 \cdot 10^{19}$ eV/ml. After irradiation the solutions were subjected to chromatographic investigation. Qualitative tests showed that solutions irradiated for from 1 to 60 min, according to the chromatographic data, practically contain no polymer.

Quantitative Determination of Amino Sugars

To obtain quantitative data on the action of radiation on glucosamine it was necessary to have a method for the quantitative determination of amino sugars without separating them from other products and suitable for determining small amounts. All existing methods of quantitative analysis of amino sugars are based on their determination in solutions (2–5) and are often associated with their preliminary conversion into some derivative (6–8) or with their partial destruction (9, 10), which inevitably requires relatively large amounts of substance at an accuracy of 5–10%. In this connection we developed a micromethod for the quantitative determination of amino sugars using paper chromatography, suitable also for their N-acetyl derivatives.

Onto the starting line, 9 cm from the edge of the chromatogram, samples of an aqueous solution of the amino sugar are applied to the paper with the aid of

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Fig. 1. Calibration curves of the dependence of the optical density of the eluate on the amount (in γ) of glucosamine (1—4) and its N-acetyl derivative (5—7) in the sample on the chromatogram for various experiments

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a specially calibrated micropipette at a distance of 3 cm from one another and dried in a stream of slightly warm air. Each spot is duplicated once or twice. For the calibration curve, the spots are necessarily applied on the same sheet of paper. Chromatographic separation is carried out by the descending method for 20-40 hr with a mixture of butanol—pyridine—benzene—water (5 : 3 : 1 : 3) or ethanol—water (95 : 5). The chromatogram is dried for 30-40 min in air and treated by the Morgan—Elson method (2). The colored spots of the amino sugar are cut out of the chromatogram in the form of identical rectangles measuring 3×5 cm, which are then cut into thinner strips and eluted with 5-6 ml of 50% acetic acid—

the lots. For complete elution of N-acetylglucosamine, 2 hours is sufficient; glucosamine must be left overnight. An aliquot of the eluate is diluted 1:10 with 50% acetic acid, and the optical density is measured on an SF-4 instrument at the corresponding maximum, 354 mμ for glucosamine and 358 mμ for N-acetylglucosamine. The optical-density measurements are made relative to a standard solution obtained by eluting a section of the chromatogram of the same size, treated under the same conditions. Measurements showed that, within the range from 20 to 70 γ for glucosamine and from 10 to 60 γ for its N-acetyl derivative, the solutions obey the Lambert—Beer law. Since, when chromatograms are treated, it is impossible to reproduce twice absolutely identical spraying with reagent, heating, etc., the calibration curves obtained in different experiments do not coincide exactly with one another, but are parallel to one another. In this connection, in order to increase the accuracy of the method, proceeding from the strict proportionality between the amount of amino sugar and the optical density of the eluate, we preferred in all cases to apply to the chromatogram, simultaneously with the solution under investigation, 2-3 spots of the same amino sugar of known concentration, close to that expected in the sample under investigation. Taking into account the indicated proportionality, the concentration of the amino sugar in the product under investigation can be determined reliably with an accuracy of 3-6%.

Fig. 1. Calibration curves of the dependence of the optical density of the eluate on the amount (in γ) of glucosamine (1—4) and its N-acetyl derivative (5—7) in the sample on the chromatogram for various experiments

The curves shown in Fig. 1 show that the method developed makes it possible to determine amino sugars and their N-acetyl derivatives using 10-70 γ of substance and, probably, is also suitable for larger quantities. According to the data at our

Fig. 2. Curve of the dependence of glucosamine concentration (number of molecules per 1 ml of irradiated solution) on dose

Figure 2: Fig. 2. Curve of the dependence of glucosamine concentration (number of molecules per 1 ml of irradiated solution) on dose

Fig. 3. UV spectrum for a solution irradiated with a dose of $3 \cdot 10^{19}$ eV/ml

Figure 3: Fig. 3. UV spectrum for a solution irradiated with a dose of $3 \cdot 10^{19}$ eV/ml

disposal, this method is also suitable for determining amino sugars in mixtures with other substances. The limitation in this case is, naturally, the separation of the mixture on a paper chromatogram and the obtaining of sufficiently clear and individual spots of the amino sugar, which, obviously, determine the limits of its application.

Results of the action of γ-radiation

Study of the dependence of the change in glucosamine concentration on the applied dose showed that the initial conversion of glucosamine proceeds extremely rapidly and already after the action of a dose of $1.5 \cdot 10^{19}$ eV/ml reaches 50%. The curve of the dependence of concentration on dose, shown in Fig. 2, indicates that decomposition proceeds especially rapidly in the first minutes of irradiation, after which it slows sharply. The curve presented differs substantially in character from analogous curves for the decomposition of neutral monosaccharides ([1]). Calculation by the least-squares method shows that the decomposition yield for glucosamine amounts to several tens of units. This already exceeds by more than an order of magnitude the yield for neutral–

Fig. 2. Curve of the dependence of glucosamine concentration (number of molecules per 1 ml of irradiated solution) on dose

sugars. Further investigation of the initial portion of the curve, on the basis of a larger body of experimental material, will make it possible to refine this figure. Such a high degree of conversion of glucosamine shows that it is considerably more labile to the action of radiation than other representatives of carbohydrates, and its radiolysis evidently proceeds by a different mechanism; one of the most natural assumptions is the occurrence of a chain process.

In view of the long time required for processing each solution, we were naturally interested first of all in the question of the aftereffect. We tested the possibility of an aftereffect on solutions irradiated with a dose of $1.5 \cdot 10^{19}$ eV/ml, and showed that within the time interval from 30 min to 14 days after irradiation no aftereffect is observed.

Fig. 3. UV spectrum for a solution irradiated with a dose of $3 \cdot 10^{19}$ eV/ml

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Fig. 4. Curve of the dependence of optical density on dose

Figure 4: Fig. 4. Curve of the dependence of optical density on dose

Of great interest is the fact that, beginning with a dose of $4.5 \cdot 10^{19}$ eV/ml, the character of the radiolysis process changes sharply, and with an increase in dose to $18 \cdot 10^{19}$ eV/ml the concentration of glucosamine decreases only very little. This could be explained by a change in the physical conditions during radiolysis (increase in temperature, change in pH); however, investigations showed that the aqueous solution of glucosamine is stable to the insignificant and short-term fluctuations in temperature that occur in our case, and that the pH of the solution during radiolysis remains practically constant and is about 8.63 as the dose is varied from 0 to $18 \cdot 10^{19}$ eV/ml. It may be assumed that in the process of radiolysis of glucosamine a compound is formed that competes with glucosamine or inhibits the possible chain process. Indeed, chromatographic investigation showed that during the radiolysis of glucosamine a compound is formed which is separated with difficulty on the chromatogram in the ethanol —water system (95 : 5) from glucosamine and is developed by the Morgan—Elson reagent upon 10-minute heating at 105° as a yellow spot. It is also well developed by silver nitrate and periodate. The intensity of the coloration on the chromatogram increases with increasing dose.

Fig. 4. Curve of the dependence of optical density on dose

The UV spectrum of the irradiated solutions, shown in Fig. 3, has an absorption maximum in the region of 268—270 mμ, which is characteristic of diacetone or other compounds containing an enediol grouping. As can be seen from Fig. 4, the value of the optical density at 268—270 mμ increases intensively in proportion to the dose, and when the dose is varied from $0.3 \cdot 10^{19}$ eV/ml to $18 \cdot 10^{19}$ eV/ml it increases 15-fold. It should be noted that such an increase in absorption in the region where the destruction of glucosamine practically stops agrees well with the assumption of the formation of radical acceptors more active than glucosamine.

Preliminary experiments carried out using an electron accelerator as the radiation source show that in this case the radiolysis of glucosamine proceeds differently.

A number of features of the action of radiation on amino sugars, distinguishing them from neutral sugars, and especially the rapidity of their destruction at the first stage, make further, more detailed study of the radiation chemistry of amino sugars very interesting; work in this direction is continuing.

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REFERENCES

1. G. O. Phillips, *Adv. in Carbohydrate Chem.*, **16**, 13 (1961).
2. L. A. Elson, W. T. J. Morgan, *Biochem. J.*, **27**, 1824 (1933).
3. S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).
4. D. Aminoff, W. T. J. Morgan, W. M. Watkins, *Biochem. J.*, **46**, 426 (1950).
5. E. F. Annison, A. T. James, W. T. J. Morgan, *Biochem. J.*, **48**, 477 (1951).
6. D. Aminoff, W. T. J. Morgan, *Nature*, **162**, 579 (1948).
7. P. W. Kent, G. J. Lawson, A. Semor, *Science*, **113**, 354 (1951).
8. S. Leskowitz, E. Kabat, *J. Am. Chem. Soc.*, **76**, 4887 (1954).
9. S. Gardell, F. Heijkenskjold, A. Rochnorlund, *Acta Chem. Scand.*, **4**, 970 (1950).
10. Lee Yuan, R. Montgomery, *Arch. Biochem. and Biophys.*, **93**, 2, 232 (1961).

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