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Enzymatic Synthesis of 5-Bromo-2'-deoxyuridine-2-^{14}C and of 5-Iodo-2'-deoxyuridine-2-^{14}C and their Incorporation into Deoxyribonucleic Acid (*Allium cepa*)

V. Fučík* and J. Kára**

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Praha

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Abstract. 5-Bromo-2'-deoxyuridine-2-^{14}C was prepared from 5-bromouracil-2-^{14}C and 2'-deoxyguanosine using trans-N-deoxyribosylase from *Lactobacillus helveticus* and incorporated into DNA of *Allium cepa* roots. After isolating the DNA and hydrolyzing it enzymatically to deoxynucleoside-5'-phosphates a radioactive nucleotide was detected which yielded 5-bromo-2'-deoxyuridine-2-^{14}C on enzymatic dephosphorylation. The incorporation of 5-iodo-2'-deoxyuridine-2-^{14}C was followed only by microautoradiography.

Uracil derivatives halogenated in the 5 position are incorporated into nucleic acids of various organisms. The incorporation of 5-bromouracil into deoxyribonucleic acid (DNA) of bacteriophage was demonstrated in 1954 (Dunn, Smith 1954); it is known that 5-chloro, 5-bromo and 5-iodouracil are incorporated into bacterial DNA (Zamenhof, Reiner, Degiovani, Rich 1956) and their 2'-deoxyribosides into animal cell DNA (Prusoff 1959, Eidinoff, Cheong, Rich 1959, Erickson, Szybalski 1961). The incorporation of such halogenated bases into DNA can result e.g. in genetic mutations (Freese 1959) and in sensitization of cells to the effect of ionizing and ultraviolet radiation (Erickson, Szybalski 1961, Kaplan, Smith, Tomlin 1961.)

Metabolism of halogenated uracil derivatives has not been studied for a long time on plant material. No incorporation of 5-bromouracil as the base applied by infiltration could be demonstrated in the DNA of *Cucumis sativus* (Šebesta, Bauerová, Šorm, Šormová 1960). In the course of this work a paper appeared (Smith, Kugelman, Commerford, Szybalski 1963) dealing with the incorporation of 5-iodo-2'-deoxyuridine into the DNA of *Vicia faba.*

* *Address:* Na cvičišti 2, Praha 6

** *Present Address:* Institute of Experimental Biology and Genetics, Na cvičišti 2, Praha 6

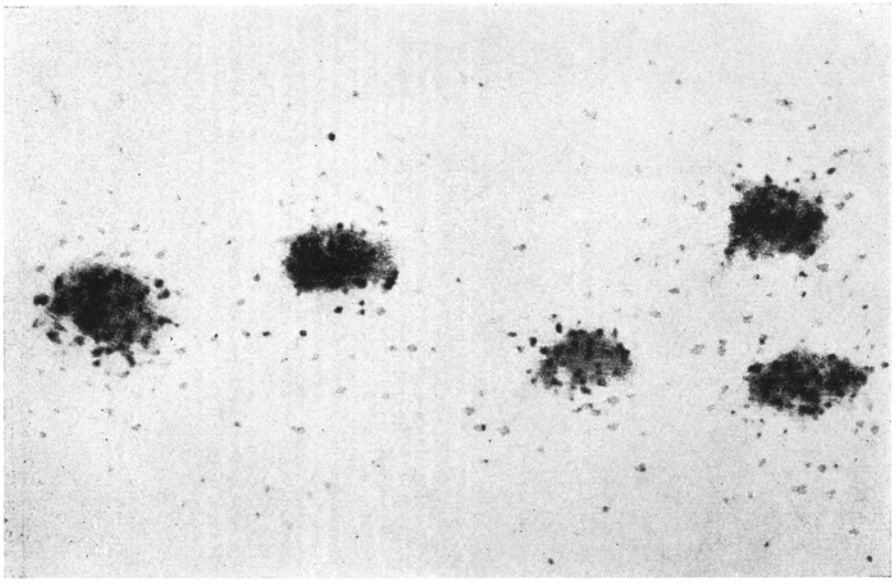

Fig. 1. Microautoradiography of cells from the root meristem of germinating seeds of *Allium cepa*. Stained with Feulgen's nucleal reaction.

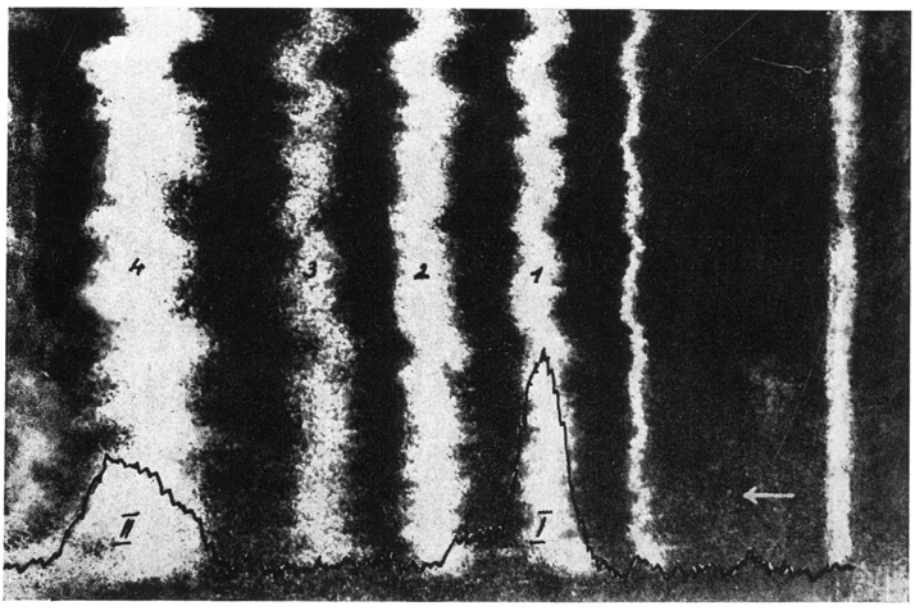

Fig. 2. Photoreprint in UV light of the chromatogram of enzymatic DNA hydrolyzate with incorporated 5-bromo-2′-deoxyuridine-2-^{14}C. Separated in isobutyric acid-ammonia-water. Radioactive zone I: 5-bromo-2′-deoxyuridine-5′-phosphate-2-^{14}C. UV-absorbing zones: 1 deoxyguanylic acid, 2 deoxythymidylic acid, 3 deoxycytidylic acid, 4 deoxyadenylic acid. Direction of chromatographic separation is indicated by the arrow. The graphical record corresponds to radioactivity.

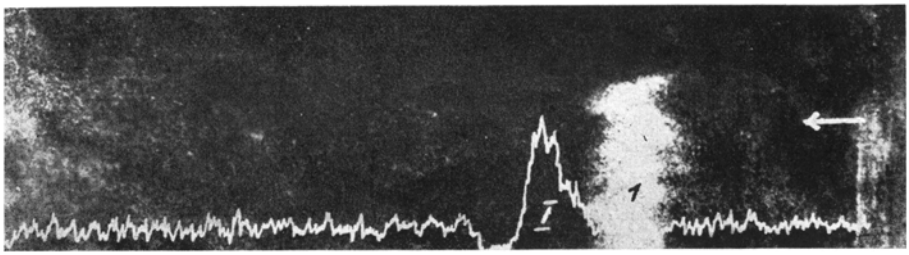

Fig. 3. Chromatographic separation of 5-bromo-2′-deoxyuridine-5′-phosphate-2-^{14}C (I) from deoxyguanylic acid. Separated in isopropanol-water (7 : 3).

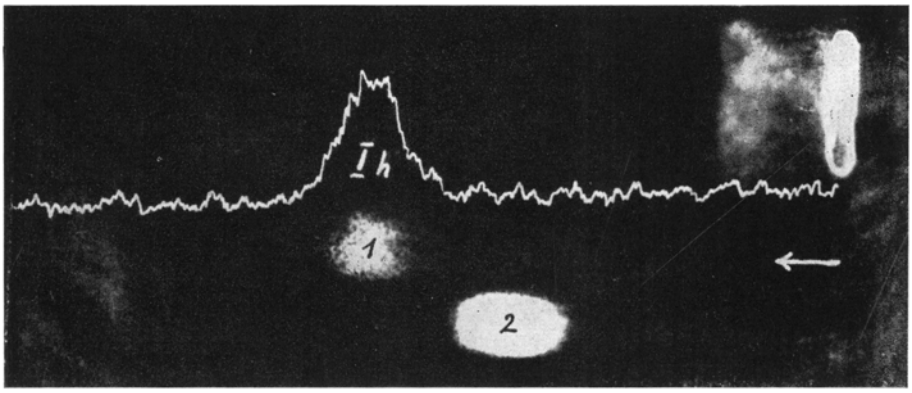

Fig. 4. Chromatography of 5-bromo-2′-deoxyuridine-2-^{14}C (Ih) formed by enzymatic dephosphorylation of the nucleotide (I in Fig. 3) and standards of 5-bromo-2′-deoxyuridine (1) and 2′-deoxyuridine (2).

The present communication presents experimental evidence about the incorporation of radioactive bromodeoxyuridine and iododeoxyuridine into the DNA of *Allium cepa* roots.

Material, Methods and Results

Preparation of reagents. 5-Bromo-2'-deoxyuridine-2-^{14}C was prepared by enzymatic deoxyribosylation of 5-bromouracil-2-^{14}C using trans-N-deoxyribosylase isolated from *Lactobacillus helveticus* (KÁRA, ŠORM 1963). 5-Bromouracil-2-^{14}C (100 μC, 1·9 mg.) and 2'-deoxyguanosine (2 mg.) were dissolved in 1·1 ml. distilled water and 0·1 ml. 1 M glycylglycine buffer of pH 7·4 and 1·5 ml. of the trans-N-deoxyribosylase (12 mg. protein) added. The mixture was incubated at 37°C for 18 h. Deproteinization was carried out by adding excess ethanol (7 volumes) and the precipitated protein centrifuged. The supernatant was evaporated under an IR lamp to 0·5 ml. and resolved by chromatography on Whatman no. 1 paper in butanol-acetic acid-water (71 : 7 : 22). 5-Bromouracil-2-^{14}C and 5-bromo-2'-deoxyuridine-2-^{14}C which have similar R_F values in the solvent system used were cut out from the paper and rechromatographed in isopropanol-water (7 : 3). In this system the two substances were well separated (R_F of bromouracil is 0·65, that of 5-bromo-2'-deoxyuridine 0·73). 5-Bromo-2'- deoxyuridine-2-^{14}C was identified on the chromatogram according to its UV absorption, its radioactivity and by estimating deoxyribose (BUCHANAN 1951). The yield of 5-bromo-2'-deoxyuridine-2-^{14}C amounted to about 10% of the total radioactivity of 5-bromouracil-2-^{14}C.

5-Iodo-2'-deoxyuridine-2-^{14}C was prepared by iodination of deoxyuridine-2-^{14}C (KÁRA, ŠORM 1963) according to PRUSOFF (1960).

Incorporation of 5-iodo-2'-deoxyuridine. The germinating seeds of onion (cultivar Všetatská) were placed on the bottom of a flask 2 cm in diameter and containing 5-iodo-2'-deoxyuridine-2-^{14}C of total activity amounting to 516 000 counts/min. The incubation lasted for 24 h. at room temperature. The root tips were fixed by a mixture of ethanol and acetic acid (3 : 1) and embedded in paraffin. Microscopic sections 5 μ in thickness were then prepared. After removing the paraffin the preparations were hydrolyzed for 10 min. at 60°C in 1N-HCl and stained with the Feulgen nucleal reaction. After washing in sulphite and distilled water the preparations were covered with a film stripped off the KODAK AR 10 plates and exposed for 1 month. A 1 : 10 diluted Rodinal developer was used for developing the preparations.

As shown in Fig. 1 the radioactivity is selectively accumulated in cell nuclei. Mitotic activity ceased during the experiment so that the photograph shows only interphase nuclei.

Incorporation of 5-bromo-2'-deoxyuridine. A solution of 5-bromo-2'-deoxyuridine-2-^{14}C (840 000 counts/min./ml.) was divided into two flasks of 3·5 ml. volume, onto which two peeled onions about 1·5 cm. in diameter were placed. The onion roots grew in the solution of radioactive bromodeoxyuridine which was daily made up with water to the original volume. Incubation took place in the light and at room temperature. After 6 days the roots were severed (2·1 g.), combined with roots growing in water alone (8 g.) and DNA isolated by a modification of the method of DOSKOČIL (1963).

Preparation of DNA. The roots were homogenized in acetone precooled with solid carbon dioxide to −60°C. The homogenate was centrifuged at 3000 r.p.m. for 5 min. and the sediment washed three times with cold acetone. After drying in air it was suspended in 15 ml. 0·15M NaCl and 0·1M ethylenediamine tetraacetic acid (the pH of the solution was previously adjusted with NaOH to 8·5). The suspension was stirred with 1·5 ml. dodecyl sulphate (5% in 45% ethanol) for 3 h. Sodium chloride was then added to a 1M concentration and the suspension centrifuged at 15 000 r.p.m. for 30 min. The supernatant was precipitated with alcohol, the precipitate centrifuged, dissolved in 15 ml. 0·15M NaCl with 0·15M sodium citrate and again mixed with dodecyl sulphate and stirred for 3 h. After making up the concentration of sodium chloride to 1M and centrifuging the supernatant was precipitated with ethanol, fibrous DNA coiled on a glass rod was dissolved in 5 ml. 1·15M NaCl with 0·015M sodium citrate. The admixture of ribonucleic acid was removed by digestion with ribonuclease (MARMUR 1961).

Hydrolysis of DNA. DNA was hydrolyzed to the deoxynucleotides with pancreatic deoxyribonuclease and snake phosphodiesterase. For this reason the DNA was precipitated with ethanol

after hydrolysis with ribonuclease and dissolved in 2 ml. 0·01M tris-HCl buffer (pH 7·4) and 0·1 ml. 0·15M $MgSO_4$ added. The cleavage was effected by adding 1 mg. deoxyribonuclease at 37°C for 2 h. After this incubation the solution was heated for 1 min. to 100°C and after adding 0·2 ml. 1M tris-HCl buffer of pH 8·5 and 1 ml. (0·8 mg.) phosphodiesterase (of Russel's viper venom) (HURST, BUTLER 1951) incubated for 18. h. at 37°C. After incubation the mixture was placed directly without previous deproteinization on Whatman no. 1.

Separation of deoxyribonucleotides. Deoxyribonucleotides were separated chromatographically for 24 h. in isobutyric acid-ammonia-water (66 : 1·5 : 33) (MAGASANIK, VISCHER, DONIGER, ELSON, CHARGAFF 1950) and the radioactivity on the chromatogram assayed on an automatic scaler. Radioactivity counts were superimposed into the copy of the chromatogram obtained in UV light (Fig. 2).

Radioactivity was found in two zones (I and II) absorbing in UV light. Zone I had the same R_F in the given solvent system as deoxyguanylic acid, zone II coincided with deoxyadenylic acid. After rechromatography of zone I in isopropanol-water (7 : 3) a radioactive zone separated from the zone of deoxyguanylic acid, displaying no UV absorption apparently on account of its small concentration (Fig. 3). Since no standard of 5-bromo-2'-deoxyuridylic acid was at our disposal the radioactive nucleotide (zone I) was hydrolyzed by 5'-nucleotidase from Russel's viper venom. The radioactive deoxyriboside formed had the same R_F value in butanol-acetic acid-water (71 : 7 : 22) as did 5-bromodeoxyuridine (Fig. 4). Similarly, 5-bromodeoxyuridine (zone II, Fig. 2) separated in this system from deoxyadenylic acid, the 5-bromo derivative having been formed apparently by an admixture of 5'-nucleotidase in the phosphodiesterase preparation.

Discussior

It follows from the analysis of DNA that the radioactive deoxynucleotide in the enzymatic hydrolyzate (zone I, Fig. 2) is identical with 5-bromo-2'-deoxyuridine-5'-phosphate formed from the administered 5-bromo-2'-deoxyuridine-2-^{14}C. Similarly 5-iodo-2'-deoxyuridine-2-^{14}C is incorporated into cell nuclei as shown by the results of microautoradiography in agreement with the results obtained by SMITH, KUGELMAN and COMMERFORD (1963). According to these authors 5-iododeoxyuridine replaced as much as 20% thymidine in the DNA of *Vicia faba*. In view of the small amount of material in our experiments it was not possible to evaluate quantitatively the percentage of incorporated analogues in DNA. It follows from the results that 5-bromo-2'-deoxyuridine is incorporated into plant DNA in measurable quantities while incorporation of 5-bromouracil into plant DNA could not be demonstrated even qualitatively. It is possible that 5-bromouracil is mostly catabolized by plant cells (ŠEBESTA, BAUEROVÁ, ŠORM, ŠORMOVÁ 1960) while 5-bromo-2'-deoxyuridine is phosphorylated by the kinases to 5-bromo-2'-deoxyuridine-5'-triphosphate which can be polymerized during DNA synthesis (BESSMAN, LEHMAN, ADLER, ZIMMERMAN, SIMMS, KORNBERG 1958).

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V. FUČÍK, J. KÁRA, Ústav organické chemie a biochemie, Československá akademie věd, Praha: **Enzymatická syntéza 5-bromo-2'-desoxyuridinu-2-^{14}C a 5-jodo-2'-desoxyuridinu-2-^{14}C a jejich inkorporace do desoxyribonukleové kyseliny** *Allium cepa*. — Biol. Plant. 6 : 232—235, 1964.

5-Brom-2'-desoxyuridin-2-^{14}C byl připraven z 5-bromuracilu-2-^{14}C a 2'-desoxyguanosinu za použití trans-N-desoxyribosylázy z *Lactobacillus helveticus* a inkorporován do DNK v kořenech *Allium cepa*. Po izolaci DNK a její enzymové hydrolýze na desoxynukleosid-5'-fosfáty byl zjištěn radioaktivní nukleotid, jehož enzymovou defosforylací byl získán 5-bromo-2'-desoxyuridin-2-^{14}C. Inkorporace 5-jodo-2'-desoxyuridinu-2-^{14}C byla sledována pouze mikroautoradiografií.

В. ФУЧИК, И. КАРА, Институт органической химии и биохимии ЧСАН, Прага: **Энзиматический синтез 5-бром-2'-дезоксиуридина-2-^{14}C и 5-йод-2'-дезоксиуридина-2-^{14}C и их инкорпорация в дезоксирибонуклеиновую кислоту** *Allium cepa* L. — Biol. Plant. 6 : 232—235, 1964.

5-бром-2'-дезоксиуридин-2-^{14}C приготовлен из 5-бромурацила-2-^{14}C и 2'-дезоксигуанозина с использованием транс-N-дезоксирибозилазы из *Lactobacillus helveticus* и инкорпорирован в ДНК в корнях *Allium cepa*. После изоляции ДНК и ее энзиматического гидролиза на дезоксинуклеозид-5'-фосфаты установлен радиоактивный нуклеотид, энзиматической дефосфориляцией которого получен 5-бром-2'-дезоксиуридин-2-^{14}C. Инкорпорация 5-йод-2'-дезоксиуридина-2-^{14}C изучалась путем микроавторадиографии.