GLASNIK INSTITUTA ZA BOTANIKU I BOTANICKE BAŠTE UNIVERZITETA U BEOGRADU
BULLETIN DE L'INSTITUT ET DU JARDIN BOTANIQUES DE L'UNIVERSITÉ DE BEOGRAD
Tom XI nov. ser., 1—4, 1976, Beograd

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OROTIC ACID UTILIZATION FOR THE LABELLING OF NUCLEIC ACIDS IN THE WHEAT SEEDLINGS

INTRODUCTION

Since it was introduced by Hurlbert and Potter in 1952, radioactive orotic acid has been extensively used as labelled precursor of nucleic acids in different systems, especially in long-term metabolic studies. We have been interested to check for the possibility of usage of this precursor in short-term metabolic studies of nucleic acids biosynthesis in wheat seedlings. In this paper we present data showing that orotic-6-^{14}C acid could be used as an efficient label of all nucleic acid classes in wheat seedlings after relatively short labelling periods.

MATERIAL AND METHODS

In all experiments dry wheat seeds (*Triticum vulgare* L.), harvest 1972 were used.

Cultures were grown in the dark at 27°C. Each experimental group consisted of one hundred seeds placed on filter paper moistened with water in covered Petri dishes. The seedlings have been harvested after 48 hrs of seed imbibition and at specified intervals after addition of orotic-6-^{14}C acid.

No bacterial or fungal growth was apparent during the incubation period.

Total nucleic acids from 48 hrs old seedlings have been isolated according to Petrović *et al.* (1966) using mortar and pestle for homogenization. Chromatography of nucleic acids on methylated albumin-kieselghur (MAK) columns was performed by stepwise elution as described by Sueoka and Cheng (1962). Radioactivities of nucleic acid fractions separated by MAK chromatography were measured in Beckman L.S. 150 scintillation counter, using Bray's (1960) scintillation liquid.

For determination of specific radioactivites, nucleic acids were separated by salt fractionation (Wicks *et al.*, 1965; Greenman

et al., 1965) and precipitated with trichloracetic acid (TCA) after addition of unlabelled ribonucleic acid (RNA) as a carrier. The precipitates were collected by vacuum filtration on membrane filters (Schleicher and Schuel, B-6) and washed exhaustively with cold TCA. The washed filters were dried and counted in toluene scintillation medium.

Orotic-6-^{14}C acid (spec. act. 34.2 mCi/mM) has been purchased from »Boris Kidrič« Institute, Vinča, Beograd.

All chemicals used throughout this work were analytical reagent grade.

EXPERIMENTAL RESULTS

Fig. 1 represents elution profiles obtained by MAK chromatography of total nucleic acids extracted from 48 hrs old wheat seedlings. The seeds imbibed in 7.5 ml of destilled water containing 20 μCi of labelled orotic acid for the first 24 hrs. After that, another 20 μCi of the same precursor were added in 0.1 ml of water. 24 hrs later, nucleic acids were isolated and analyzed either by means of MAK chromatography or by salt fractionation as described under Methods.

From radioactivity and optical density profiles (Fig. 1) as well as from specific radioactivities of different nucleic acid classes (Table 1) it is obvious that the labelled precursor was very efficiently utilized for the labelling of nucleic acids.

Table 1. — Specific radioactivities of nucleic acids isolated from wheat seedlings different time after application of orotic-6-^{14}C acid

Specifične radioaktivnosti nukleinskih kiselina izolovanih iz klica pšenice različito vreme po dodavanju orotinske-6-^{14}C kiseline

Duration of orotic acid incorporation	s-RNA	Cts. (min.) mg DNA	HMW-RNA	Ten. bound component
Dužina obeležavanja orotinskom kiselinom	s-RNK	Otkucaji (*n.in.*) mg DNK	VM-RNK	Ireverzibilno vezana komponenta
48 hrs	3,747.000	4,726.000	6.044.000	78.000
24 hrs	506.400	520.400	475.270	43.680
2 hrs	130.000	76.350	84.910	68.890
2 hrs*	126.700	115.950	32.480	48.500

* Seedling have been collected 46 hrs after beginning of imbibition and orotic-6-^{14}C acid has been added for the next 2 hrs.

* Klice stare 46 h su odsecane i na njih je dodavana obeležena orotinska kiselina koja je ugrađivana u nukleinske kiseline tokom 2 h.

Similar picture has been obtained for 24 hrs labelling period, 20 μCi of orotic-6-^{14}C acid being added to the cultures 24 hrs after imbibition in 7.5 ml of water (Fig. 2).

As seen from Fig. 2 and from numerical values for specific radioactivities of different nucleic acid classes (Table 1) including tenaci-

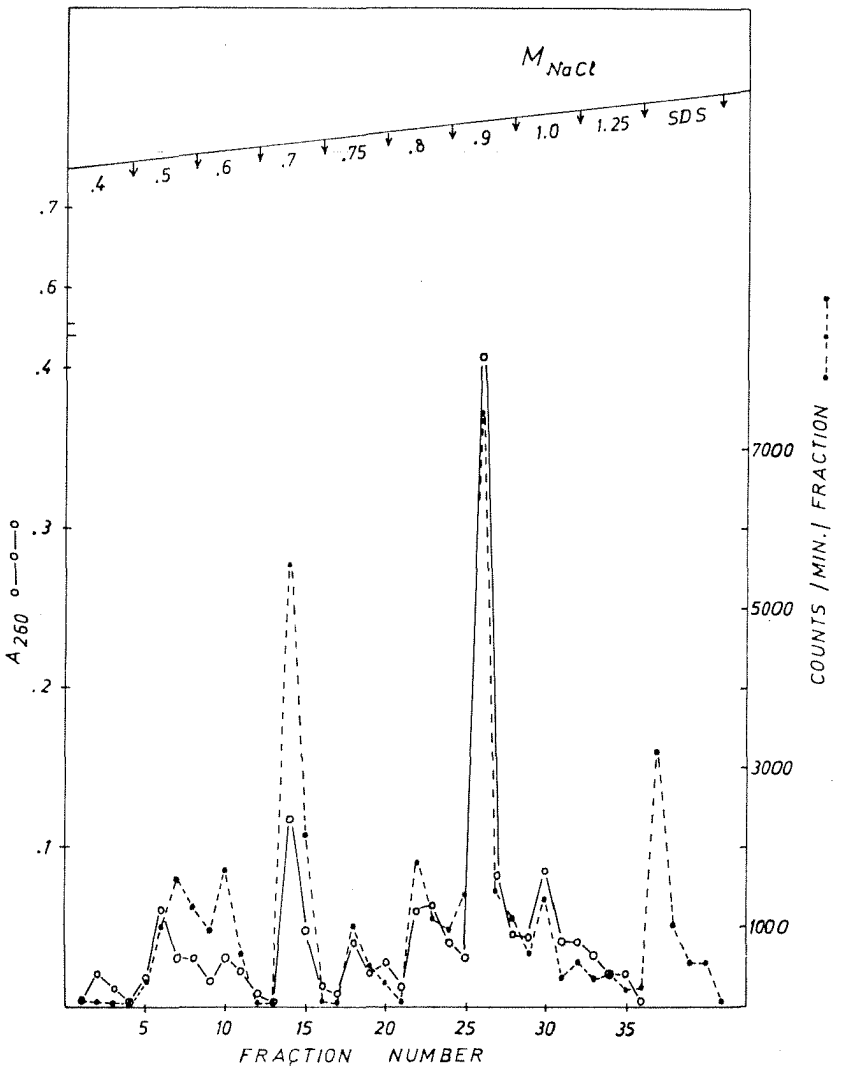

Fig. 1. — Elution profiles of total wheat seedling nucleic acids upon MAK chromatography. The seeds were imbibed in 7.5 ml of destilled water containing 20 μCi of orotic-6-^{14}C acid for the first 24 hrs of germination, and then another 20 μCi of the same precursor have been added for the next 24 hrs. The column was loaded with 1 mg of nucleic acids which were eluted by step-wise elution with NaCl solution (0.4 — 1.25 M); 5 ml fractions were collected.

Elucioni profili ukupnih nukleinskih kiselina klica pšenice dobijeni MAK hromatografijom. Seme je bubrilo u 7.5 ml destilovane vode koja je sadržala 20 μCi orotinske-6-^{14}C kiseline. Posle prva 24 h klijanja dodavano je još 20 μCi istog obeleživa-ča. Ukupne nukleinske kiseline su izolovane 24 h posle drugog dodavanja radioaktivnog markera. Na kolonu je nanošen 1 mg nukleinskih kiselina koje su eluirane diskontinuiranim gradijentom NaCl (0.4 — 1.25 M). Prikupljane su frakcije od 5 ml.

ously bound component eluted from the column with hot 1% sodium dodecyl sulphate solution (E l l e m, 1966) radioactive precursor has been very efficiently incorporated into these macromolecules under the the experimental conditions applied.

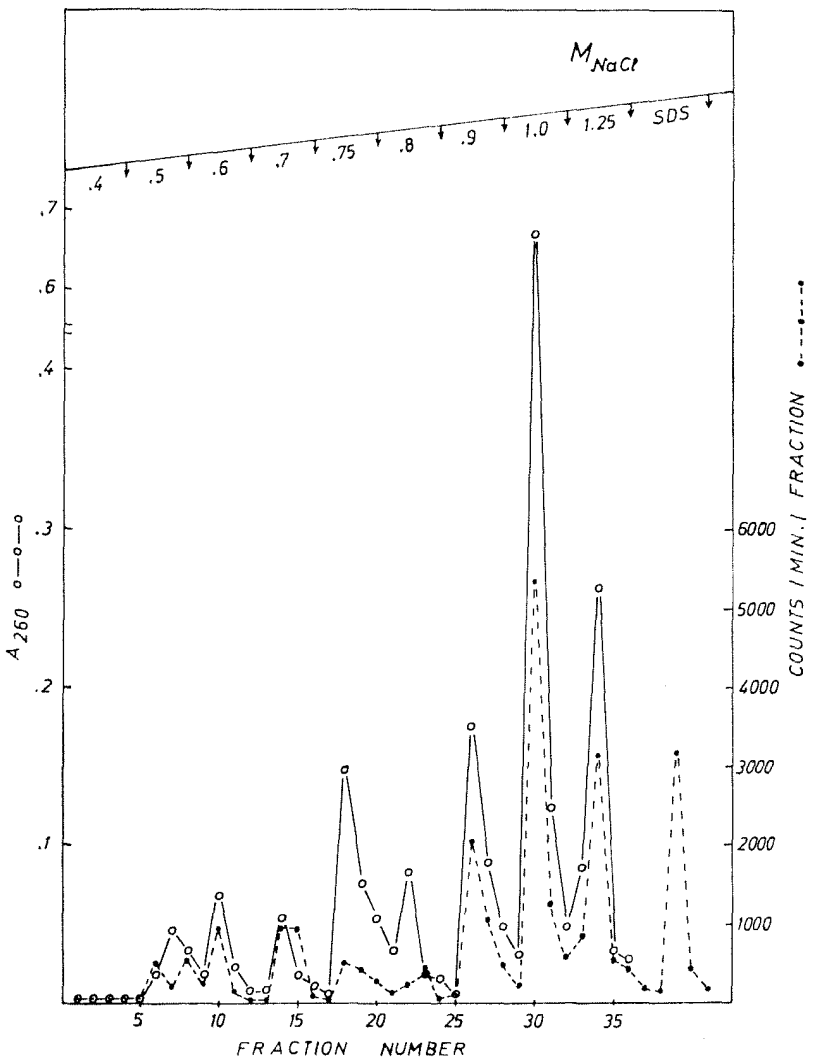

Fig. 2. — Elution profiles of total wheat seedling nucleic acids obtained by MAK chromatography. Labelled orotic acid (20 μCi) has been added to the cultures 24 hrs after beginning of imbibition, incorporation period being 24 hrs. Total nucleic acids have been analysed as described in legend to Fig. 1.

MAK hromatogrami ukupnih nukleinskih kiselina klica pšenice. Obeležena orotinska kiselina (20 μCi) je dodavana u kulture 24 h posle početka bubrenja. Nukleinske kiseline su izolovane 24 h posle dodavanja obeleživača. Uslovi MAK hromatografije su bili isti kao što je opisano u legendi uz sl. 1.

Fig. 3 represents MAK chromatographic profiles of nucleic acids isolated from 48 hrs old wheat seedlings, labelling period being 2 hrs only.

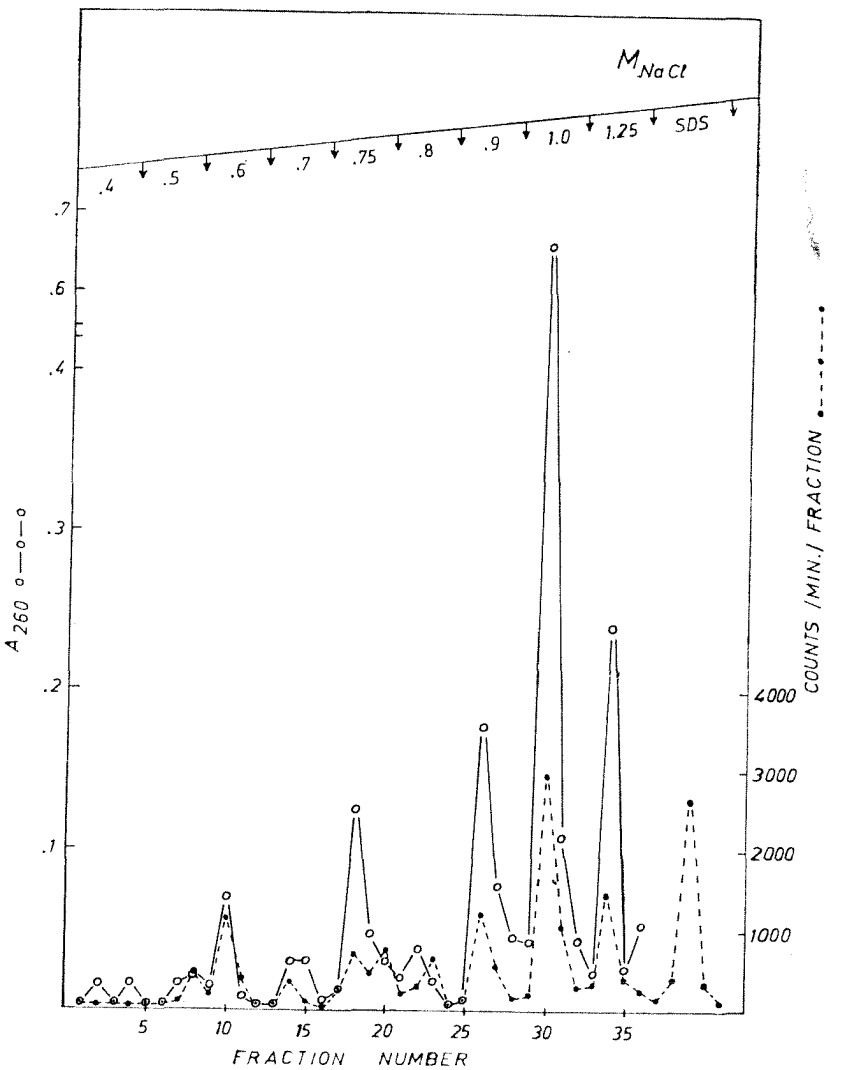

Fig. 3. — Elution profiles of total wheat seedling nucleic acids upon MAK chromatography. Orotic-6-^{14}C acid (20 μCi) has been added to the cultures 46 hrs after beginning of imbibition, labelling period being 2 hrs only. Chromatographic analysis has been performed as described in the legend to Fig. 1.

Elucioni profili ukupnih nukleinskih kiselina klica pšenice dobijeni MAK hromatografijom. U kulture je dodavano 20 μCi orotinske-6-^{14}C kiseline 46 h posle početka bubrenja. Obeležavanje je trajalo samo 2 h. Hromatografska analiza je rađena kako je opisano u legendi uz sl. 1.

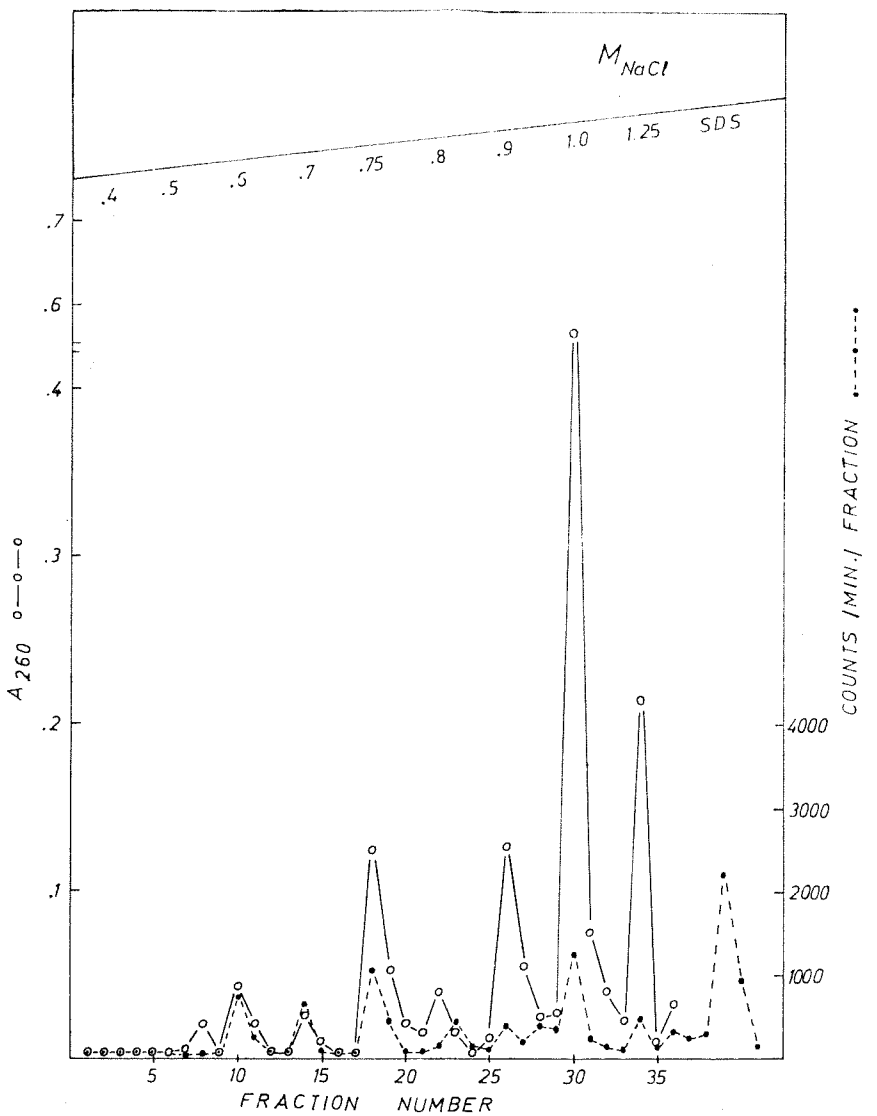

Fig. 4. — Elution profiles of total nucleic acids isolated from 48 hr old wheat seedlings which have been collected 2 hrs before the application of labelled orotic acid (20 μCi) and analyzed by means of MAK chromatography. Chromatographic analysis has been done as depicted in the legend to Fig. 1.

Elucioni profili ukupnih nukleinskih kiselina klica pšenice dobijeni MAK hromatografijom. Klice stare 46 h su odsecane i na njih je dodavano 20 μCi obeležene orotinske kiseline. Nukleinske kiseline su ekstrahovane 2 h posle dodavanja radioaktivnog obeleživača i analizirane MAK hromatografijom kako je opisano u legendi uz sl. 1.

From optical density and radioactivity profiles as well as from specific radioactivities (Table 1) of different nucleic acid classes it can be seen that even relatively short labelling period of 2 hrs is long enough for an efficient labelling of wheat nucleic acids.

In another series of experiments seedlings were harvested after 46 hrs of germination and labelled orotic acid has been added (20 μCi/1 g of seedlings) for the next 2 hrs. Nucleic acids have been isolatedm as described under Methods and separated either by MAK chromatography or salt fractionation. Elution profiles after MAK chromatography are depicted on Fig. 4 and numerical values for specific radioactivities of different nucleic acid classes are given in Table 1.

It is obvious (Fig. 4, Table 1) that orotic-6-^{14}C acid can be used as an radioactive label of all nucleic acid classes in wheat seedlings after they have been harvested.

DISCUSSION

The choice of radioactive precursor for the labelling of cellular nucleic acids is a serious problem for investigator. For the labelling of these macromolecules inorganic phosphate (—$^{32}PO_4^{-3}$) has been often used as radioactive label in short-term metabolic studies. It incorporates into all four nucletides thus giving high specific radioactivities of nucleic acid samples, but disadvantages of its usage are numerous, e.g. contamination of saples with inorganic radioactive molecules, its incorporation into different compounds, short life-time of ^{32}P itself etc. Our results presented in this paper show that labelled orotic acid can be effectively used for the labelling of plant nucleic acids even for the short-term metabolic studies. Specific radioactivities of different classes of nucleic acids even in the shortest labelling period studied (2 hrs) are high suggesting that in this system orotic-6-^{14}C acid could be used by all means for pulse-labelling investigations. Different classes of nucleic acids labelled as described in this paper and separated by means of MAK chromatography could be used for further analysis with or without addition of unlabelled carrier. We hope therefore that the data presented here will point the way for the much greater use of orotic acid as label in biochemistry of plant nucleic acids.

SUMMARU

Orotic-6-^{14}C acid can be used for the labelling of all classes of nucleic acids during first 48 hrs of wheat germination.

Specific radioactivities of different classes of nucleic acids separated either by means of MAK chromatography or salt fractionation even in the shortest labelling period studied (2 hrs) are relatively high showing that orotic acid can be effectively used in short-term metabolic studies of nucleic acids biosynthesis in wheat seedlings.

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Rezime

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KORIŠĆENJE OROTINSKE-6-^{14}C KISELINE ZA OBELEŽAVANJE NUKLEINSKIH KISELINA KLICA PŠENICE

Praćenjem obeležavanja pojedinih klasa nukleinskih kiselina izolovanih iz 48 h starih klica pšenice u različitim uslovima utvrđeno je da se orotinska-6-^{14}C kiselina može uspešno koristiti za metabolička proučavanja vezana za ovu klasu makromolekula. Specifične radioaktivnosti pojedinih klasa nukleinskih kiselina razdvojenih hromatografijom na kolonama metilovanog albumina — kizelgura (MAK) ili frakcioniranjem pomoću natrijum hlorida pokazuju da je korišćenje ovog radioaktivnog prethodnika nukleinskih kiselina veoma efikasno i pri relativno kratkom obeležavanju (2 h), bilo da se orotinska kiselina doda na intaktne ili odsečene klice.