

## Metabolism of hypoxanthine in wheat shoots

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(Received: May 23, 1974),

### Abstract

The incorporation of [ $^{14}\text{C}$ ] glycine and [8- $^{14}\text{C}$ ] hypoxanthine to some purine derivatives in overground parts of wheat seedlings was studied.

It was found that adenylic acid could be synthesized from glycine and also from free hypoxanthine and in both processes inosinic acid is an intermediate metabolite.

### INTRODUCTION

The first purine nucleotide formed "de novo" is inosine-5'-phosphate (IMP). It serves as a branch point for its conversion to the adenine and guanine compounds. This pathway was investigated in animal and microorganism material. On the other hand, purine bases may be rescued as nucleotides in several ways (Jeżewska 1961).

In plants natural adenine derivatives occur in considerable amounts, but hypoxanthine derivatives have been found in very low quantities and only in few species. Investigations of purine derivatives in higher plants show that the nucleotide pattern of various plants is changed during some physiological processes (Rybicka 1972).

In this work synthesis "de novo" of hypoxanthine as well as its interconversion to adenine compounds in cut-off wheat shoots was investigated.

### MATERIAL AND METHODS

**Plants.** Green, five-day-old wheat plants variety "Dańkowska biała" (*Triticum aestivum* ssp. *vulgare* var. *albidum*) were used throughout. The cut-off shoots were fed under the conditions described previously (Rybicka 1969).

**Radiochemicals.**  $\text{NaH}^{14}\text{CO}_3$ , [U- $^{14}\text{C}$ ] glycine, [8- $^{14}\text{C}$ ] adenine, [8- $^{14}\text{C}$ ] hypoxanthine.

**Extraction, separation and determination of purine derivatives.** The acid-soluble purine derivatives were extracted with chilled 0.3 N  $\text{HClO}_4$ , purified, separated and

determined as described previously (Rybicka 1969, 1972). Purine derivatives were hydrolyzed in 1 N HCl at 100° C for 1 hr. The radioactivity of purine bases was measured in an automatic liquid-scintillation counter Packard Tri-Carb model 3003, efficiency for  $^{14}\text{C}$  80%, with the use of Bray's scintillation liquid (Bray 1960).

## RESULTS AND DISCUSSION

Attempts to isolate all the hypoxanthine (after acid hydrolysis of its derivatives) from the acid-soluble fraction showed that its content is rather low. The yield of hypoxanthine is about 0.2  $\mu\text{mole}$  per 100 g of fresh cut-off wheat shoots.

Incubation of 10 g samples of cut-off wheat shoots with 0.02 mCi  $\text{NaH}^{14}\text{CO}_3$  (spec. activity 8.7 mCi/mM) for 5 min allowed the comparison of the radioactivity of adenine and hypoxanthine nucleotides (AMP and IMP). During analysis IMP carrier was added. The radioactivities of purine bases arising from AMP and IMP differ considerably: for adenine it is 90 c. p. m. and for hypoxanthine 640 c. p. m., this suggesting that IMP is the first biosynthetic product.

The synthesis of hypoxanthine and IMP in cut-off wheat shoots from  $[\text{U-}^{14}\text{C}]$  glycine was investigated after 5, 15, and 45 min of incubation (Table 1). Because of the low content of these compounds the isolation of these substances was difficult. For this reason non-labelled carriers of hypoxanthine and IMP were added to the perchloric acid extracts derived from wheat shoots fed with  $^{14}\text{C}$ -glycine. Both compounds isolated in this way were radioactive. Their radioactivity increased with the feeding time 3-fold between 5 and 15 min of incubation but the total activity of IMP was always 2 — 3 times higher than that of hypoxanthine. It suggests that IMP is the first synthesized compound. Thus, the wheat shoots synthesize during this short feeding time IMP from both radioactive precursors. IMP and hypoxanthine were found in a few plant species only, probably owing to their low quantity in the investigated plants.

Table 1

$[\text{U-}^{14}\text{C}]$  glycine incorporation into hypoxanthine and IMP in wheat shoots

Samples of wheat shoots (8g) were incubated for 5, 15 and 45 min at 20°C with 0.05  $\mu\text{mole}$  of  $[\text{U-}^{14}]$  glycine (spec. act.  $2.46 \times 10^8$  c.p.m./ $\mu\text{mole}$ ) dissolved in 1 ml of water. Each value is an average from two separately incubated samples

Incubation time (min)	Uptake* of $[\text{U-}^{14}\text{C}]$ glycine (c. p. m.)	Total radioactivity	
		Hypoxanthine (c. p. m.)	IMP** (c. p. m.)
5	$2.7 \times 10^6$	240	840
15	$5.1 \times 10^6$	750	2200
45	$6.6 \times 10^6$	1320	2800

\* Difference before and after feeding.

\*\* After hydrolysis to hypoxanthine.

In the subsequent experiments the incorporation of  $[\text{U-}^{14}\text{C}]$  glycine,  $[\text{8-}^{14}\text{C}]$  adenine and  $[\text{8-}^{14}\text{C}]$  hypoxanthine into adenine, adenosine and AMP in cut-off wheat shoots was investigated during 5 — 60 min (Table 2). The specific radioactivity of the adenine moiety from these compounds was determined. Specific activity of

Table 2

[U-<sup>14</sup>C] glycine, [8-<sup>14</sup>C] adenine and [8-<sup>14</sup>C] hypoxanthine incorporation into adenine derivatives in wheat shoots

Samples of wheat shoots (10 g) were incubated for 5, 15, 30 and 60 min at 20°C with radioactive substrates dissolved in 1 ml of water. Substrates: 0.05  $\mu$ mole of [U-<sup>14</sup>C] glycine (spec. act.  $2.46 \times 10^8$  c.p.m./ $\mu$ mole); 0.15  $\mu$ mole of [8-<sup>14</sup>C] adenine (spec. act.  $2.8 \times 10^7$  c.p.m./ $\mu$ mole); 0.50  $\mu$ mole of [8-<sup>14</sup>C] hypoxanthine (spec. act.  $2 \times 10^6$  c.p.m./ $\mu$ mole). Each value is an average from two separately incubated samples.

Radioactive substrates	Uptake* of radioactive substrates (c. p. m.)	Incubation time (min)	Adenine		Adenosine**		AMP**	
			$\mu$ mole	c. p. m. / $\mu$ mole	$\mu$ mole	c. p. m. / $\mu$ mole	$\mu$ mole	c. p. m. / $\mu$ mole
[U- <sup>14</sup> C] glycine	$5.1 \times 10^6$	15	—	10 400	—	—	—	18 000
[8- <sup>14</sup> C] adenine	$2.2 \times 10^5$	5	0.15	566 600	0.20	172 500	0.19	81 500
"	—	15	0.10	659 000	0.19	367 200	—	92 300
[ <sup>14</sup> C]hypoxanthine	$1.6 \times 10^5$	5	0.21	60	0.28	210	0.15	300
"	$2.9 \times 10^5$	15	0.23	280	0.28	1 200	0.21	1 000
"	$8.7 \times 10^5$	30	0.23	2 100	0.27	1 700	0.22	2 000
"	$9.7 \times 10^5$	60	0.23	3 200	0.20	4 100	0.19	3 500

\* Difference before and after feeding.

\*\* After hydrolysis to adenine.

AMP was considerably higher than that of adenine after 15 min of incubation with  $^{14}\text{C}$ -glycine. In the previous paper (Rybicka 1969) the differences in specific activity of adenine and AMP were not observed, maybe owing to the lower specific activity of the  $[1\text{-}^{14}\text{C}]$  glycine used.

Incorporation of  $[8\text{-}^{14}\text{C}]$  adenine into adenosine and AMP in wheat shoots during 5 and 15 min of incubation was investigated. The specific radioactivity of these compounds increases with the time of incubation. The specific activity of adenosine was twice and three times that of AMP, suggesting that exogenous adenine is converted mainly to its riboside. The results presented demonstrate that after a short time of incubation wheat shoots can convert radioactive adenine into its derivatives. Similar observations were made by Price and Murray (1969) in the germinating wheat embryo. The acid-soluble fraction was extensively labelled after incubation in the presence of  $[8\text{-}^{14}\text{C}]$  adenine. Incorporation of adenine, mostly into its derivatives by the cellular suspension of *Acer pseudoplatanus* and in hazel seeds was reported by Doree et al. (1970) and by Bradbeer and Floyd (1964). Contrary to these findings Silver and Gilmore (1969) found no products of adenine infiltrated into pea seedlings. On the other hand, adenine catabolism has been observed in leaf tissue (Barnes 1959, Schlee 1964, Schlee, Reinbothe 1965). In these experiments, even after 5 min feeding of wheat shoots with  $^{14}\text{C}$ -adenine, about one fifth of the taken up radioactivity was found in adenosine and AMP and after 15 min nearly one half.

Interconversion of  $[8\text{-}^{14}\text{C}]$  hypoxanthine into adenine derivatives was followed during 5–60 min incubation in cut-off wheat shoots. It was found that after 5 min feeding AMP exhibited the highest specific activity, it was lower in adenosine and the lowest in adenine; after 15 min feeding AMP as well as adenosine were 3–4 times more active than adenine. However, after 30 and 60 min incubation there were no noticeable differences between specific activity of adenine, adenosine and AMP arising from  $^{14}\text{C}$ -hypoxanthine. The specific activities of these adenine compounds increased with the time of incubation. Specific activity of adenine, adenosine and AMP increased 50-, 20- and 10-times, respectively from 5 min to 60 min of incubation. The results of the above experiments showed, that the interconversion of hypoxanthine into adenine derivatives occurred but in a small extent. Even after 1-hr incubation less than 1% of the activity taken up from  $^{14}\text{C}$ -hypoxanthine was found in isolated adenine compounds. The difference between specific activity of AMP and adenine arising from  $^{14}\text{C}$ -hypoxanthine could suggest, that during this process IMP is an intermediate.

To sum up, the results obtained point that AMP can arise from glycine as well as from free hypoxanthine and in both processes IMP is an intermediate.

This work was supported by the Polish Academy of Sciences within the project 09.3.1.

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*Metabolizm hipoksantyny w siewkach pszenicy*

**Streszczenie**

Badano włączanie [ $^{14}\text{C}$ ] glicyny i [8- $^{14}\text{C}$ ] hipoksantyny do niektórych pochodnych purynowych w częściach nadziemnych siewek pszenicy. Wyniki wskazują, że kwas adenylowy może być syntetyzowany z glicyny, a także z wolnej hipoksantyny i w obu procesach pośrednim produktem jest kwas inozynowy.