ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. XLI — nr 2
1972

Effect of adenine on the synthesis of purine derivatives in wheat seedlings

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(Received: November 11, 1971)

Abstract

The effect of adenine and other purine derivatives on the "de novo" purine synthesis was investigated in young wheat schoots. Adenine, adenosine and AMP inhibited strongly this biosynthesis, whereas hypoxanthine had no effect. The incorporation of each of the used precursors; [1-^{14}C] glycine, [^{14}C] formate and [^{14}C] carbonate was inhibited by adenine in the same extent.

INTRODUCTION

It is known that the biosynthesis of purines in bacteria and animal tissues is initiated by formation of 5-phosphoribosylamine which reacts then with glycine and gives glycinamide ribonucleotide. This compound is eventually converted to IMP (inosine-5'-phosphate) which is the first purine intermediate. The first reaction is regulated by a feed-back mechanism (see review by Moat and Friedman 1960). In higher plants these initial steps of purine biosynthesis were investigated by Kapor and Waygood (1962).

The aim of the present paper was to determine the effect of adenine and its derivatives on „de novo" synthesis of the purine ring in young wheat shoots. Preliminary observations on the inhibitory effect of adenine on purine ring synthesis were described previously (Rybicka 1969).

MATERIALS AND METHODS

Reagents; [1-^{14}C] glycine and $Na_2{}^{14}CO_3$ were obtained from Instytut Badań Jądrowych, Świerk, Poland. Sodium [^{14}C] formate was purchased from Radiochemical Centre, Amersham, England. All non-

labelled purine derivatives were purchased from L. Light Co., Colnbrock, England and Calbiochem, Los Angeles U.S.A. Other reagents were from Fabryka Odczynników Chemicznych, Gliwice, Poland.

Plants; Green, five-day old wheat plants variety „Dańkowska biała" (*Triticum aestivum* ssp. *vulgare* var. *albidum*) were used throughout. Feeding was carried out under conditions described previously (Rybicka 1969).

EXTRACTION, SEPARATION AND DETERMINATION OF PURINE DERIVATIVES

The acid-soluble purine derivatives were extracted with cold 0.3 N $HClO_4$ purified and separated as described previously (Rybicka 1969), with the only change in conditions of column chromatography. Dowex 1×8, 100-200 mesh, formate form, column (0.8×25 cm) was used this time. Adenine and adenosine were eluted with 150 ml of water followed by AMP (adenosine-5′-phosphate) and DPN (diphosphopyridine nucleotide) elution with 120 ml 0.4 N HCOOH. To obtain the sum of free adenine plus the adenine orginating from its derivatives (Table 1 and 3) the acidic extract from seedlings was hydrolysed at 100°C for 1 hr.

Table 1

Effect of adenine on the incorporation of [1-^{14}C]glycine, [^{14}C]formate and [^{14}C]bicarbonate into adenine derivatives

2g samples of wheat shoots were fed with 0.3 ml substrate solutions for 2 hr at 20°C. The solutions contained indicated substrates in the following quantities; [1-^{14}C]glycine, 2 760 000 c.p.m. (2 880 000 c.p.m./μmole); sodium [^{14}C]formate, 2 760 000 c.p.m. (5 280 000 c.p.m./μmole); sodium [^{14}C]bicarbonate, 1 560 000 c.p.m. (7 800 000 c.p.m./μmole); and adenine 0.2 μmole. Results are given as mean of two separate experiments

Substrates	Uptake * of radioactive substrate c.p.m.	Uptake * of adenine μmoles	Adenine ** of acid-soluble fraction		
			μmoles	c.p.m.	% of inhibition
[1-^{14}C]glycine	2 700 000	—	0.15	370	—
[1-^{14}C]glycine + +adenine	2 700 000	0.20	0.25	120	67
Sodium[^{14}C] formate	2 700 000	—	0.24	2540	—
Sodium[^{14}C] formate+adenine	2 700 000	0.15	0.23	850	66
Sodium[^{14}C] bicarbonate	1 560 000	—	0.24	170	—
Sodium[^{14}C] bicarbonate+adenine	1 560 000	0.20	0.27	60	65

* Difference before and after feeding.
** All adenine derivatives after hydrolysis.

Table 2

Effect of various concentrations of adenine on the incorporation of [1-^{14}C]glycine into individual adenine derivatives

10g samples of wheat shoots were fed with 1 ml substrate solutions for 2 hr at 20°C. The solutions contained [1-^{14}C]glycine, 8 520 000 c.p.m. (20 640 000 c.p.m./μmole) and adenine in indicated quantities. Results are given as mean of two separate experiments

Substrates	Uptake * of [1-^{14}C]glycine c.p.m.	Uptake * of adenine μmoles	Acid-soluble fraction							Acid--insoluble fraction
			Adenine		Adenosine **		AMP **			Adenine
			μmoles	c.p.m.	μmoles	c.p.m.	μmoles	c.p.m.	% of inhibition	c.p.m./μmole
[1-^{14}C]glycine	6 180 000	—	0.17	700	0.21	1890	0.24	1340	—	140
[1-^{14}C]glycine+0.2 μmole adenine	7 080 000	0.2	0.24	570	0.34	1390	0.18	450	66	80
[1-^{14}C]glycine+1.0 μmole adenine	6 660 000	1.0	0.38	680	0.26	680	0.18	250	80	40
[1-^{14}C]glycine+5.0 μmole adenine	6 000 000	4.0	1.17	410	0.60	690	0.14	115	90	30
[1-^{14}C]glycine+12.0 μmole adenine	5 700 000	8.0	4.80	240	0.30	250	0.25	65	95	30

* Difference before and after feeding.

** After hydrolysis to adenine.

The acid-insoluble fraction was hydrolysed with 1 N HCl at 100°C for 1 hr and resulting adenine was isolated and determinated as described for acid-soluble fraction.

The radioactivity of adenine was measured in an automatic liquid--scintillation counter (Packard Tri-Carb model 3003, efficiency for ^{14}C 80%) using scintillation liquid by Hall and Cocking (1965).

RESULTS

The effect of adenine on the incorporation of carbonic precursors into the purine ring was studied by feeding the cut off wheat shoots with [^{14}C] labelled substrates; [1-^{14}C] glycine, sodium [^{14}C] formate, and sodium [^{14}C] bicarbonate all mixed with non-labelled adenine (Table 1). Adenine given in the amount of 0.2 μmoles per 2g of plant tissue inhibited the incorporation of these precursors into the acid-soluble adenine derivatives by more than 60%. Adenine uptake led only to the slight increase in its content in the acid-soluble fraction.

In the subsequent experiments free adenine, adenine from adenosine, adenine from AMP and adenine from acid-insoluble fraction were determined separately. In this instance the shoots were fed with [^{14}C] glycine with non-labelled adenine in quantities from 0.2 — 12 μmoles per 10g of plant tissue (Table 2). With the increase in the amount of taken up adenine, the inhibition of glycine incorporation into AMP

Table 3

Effect of some purine derivatives on the incorporation of [1-^{14}C]glycine into adenine derivatives

2g samples of wheat shoots were fed with 0.3 ml substrate solutions for 2 hr at 20°C. The solutions contained [1-^{14}C]glycine, 1 680 000 c.p.m., (20 640 000 c.p.m./μmole) and 0.2 μmole of the indicated purine derivatives. Results are given as mean of two separate experiments

Substrates	Uptake * of [1-^{14}C]glycine c.p.m.	Uptake * of purine derivatives μmoles	Adenine ** of acid-soluble fraction		
			μmoles	c.p.m.	% of inhibition
[1-^{14}C]glycine	1 600 000	—	0.27	1190	—
[1-^{14}C]glycine + +AMP	1 600 000	0.15	0.29	290	76
[1-^{14}C]glycine + +adenosine	1 600 000	0.13	0.29	290	76
[1-^{14}C]glycine + +hypoxanthine	1 600 000	0.20	0.30	1290	0

* Difference before and after feeding.

** All adenine derivatives after hydrolysis.

grows higher and content of free adenine in plants increases. The quantitative differences clearly occur in free adenine at 1 μmole of adenine uptake and in adenosine only at the uptake of 4 μmoles of adenine. The uptake of 0.2 μmoles of adenine causes 66% inhibition of glycine incorporation into AMP. This quantity represents barely about 20% of adenine content out of all derivatives of acid-soluble fraction in 10 g of shoots. Specific activities of adenine from acid-insoluble fraction indicate also the strong inhibition of glycine incorporation along the increase of concentration of introduced adenine.

Adenosine and AMP also exert inhibitory influence on the purine ring biosynthesis (Table 3). This inhibition amounted to 76%, when 0.2 μmoles adenosine or AMP were introduced into 2g of plant tissue. No inhibitory effect of hypoxanthine was observed.

DISCUSSION

Presented results confirm previous preliminary observations concerning the possibility of purine biosynthesis inhibition by adenine in higher plants (Rybicka 1969). Adenine lowered not only the [1-^{14}C] glycine incorporation but also the incorporation of [^{14}C] formate and [^{14}C] carbonate confirming the assumption that it is the purine ring synthesis that is inhibited. Even very low concentration of adenine in the medium (0.2 mM), which did not cause any significant changes in adenine content, lowered the incorporation of [1-^{14}C] glycine into AMP by 66%. The decrease in incorporation of [1-^{14}C] glycine into the purine ring were caused also by adenosine and AMP.

Similar observatoins were made recently by Trachewsky and Johnstone (1969). In the extracts from Ehrlich ascites cells adenine in 2mM concentrations inhibited the FGAR (formylglycinamide ribotide) biosynthesis in 80%. Sato and Shiio (1970) in their investigations established the inhibitory effect of adenosine on PRPP (phosphoribosylpyrophosphate) amidotransferase from *Bacillus subtilis*. The inhibitory effect of AMP on amidotransferase from *B. subtilis* was observed by Shiio and Ishii (1969) and from *Schizosaccharomyces pombe* by Nagy (1970). The inhibition of purine ring synthesis by AMP was observed and investigated recently in the extracts from Ehrlich ascites cells by Trachewsky and Johnstone (1969), from *Escherichia coli* by Le Gal, Le Gal, Roche, Hedegaard (1967).

Experiments on wheat shoots established that adenine, adenosine and AMP caused inhibition of purine ring synthesis also in plant material. The question remains however if adenine itself inhibited the purine biosynthesis in plants as it was recently observed in extracts of Ehrlich ascites cells. The existence of free adenine pool in wheat shoots

suggests that it may play a role in a feed-back regulation in plants. The pools of adenosine and AMP in wheat and their inhibitory effect on PRPP amidotransferase suggest the same role in plants. On the other hand hypoxanthine had no effect on [1-^{14}C] glycine incorporation into purine derivatives and in 2mM concentrations hypoxanthine did not inhibit the FGAR biosynthesis in the extracts from Ehrlich ascites cells (Trachewsky and Johnstone 1969).

The role of adenine and its derivatives in the feed-back regulation of purine biosynthesis has been known previously in microorganisms and animal tissues. Taking under consideration recent works concerning the effect of these compounds on the first stage of purine biosynthesis, it is reasonable to assume that the biosynthetic mechanism of purines and its regulation may be the same in higher plants as in other organisms.

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

Wpływ adeniny na syntezę pochodnych purynowych w siewkach pszenicy

Streszczenie

Badano wpływ adeniny i innych pochodnych purynowych na syntezę pierścienia purynowego „de novo" w odciętych siewkach pszenicy. Adenina, adenozyna i AMP silnie hamowały biosyntezę, natomiast hypoksantyna pozostawała bez wpływu.

Hamowanie przez adeninę wcielania każdego z zastosowanych prekursorów; [1-^{14}C] glicyny, [^{14}C] mrówczanu i [^{14}C] węglanu było mniej więcej jednakowe.