Uracil and uridine metabolism in vitro in higher plants

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Our results published previously, concerning incorporation in vivo of uracil and uridine into RNA pyrimidines in plant material have suggested that two independent enzyme systems may be responsible for the metabolism of uracil into 5'-UMP (Wasilewska and Reifer 1967). One of them would be based on ribosidation of uracil to uridine in presence of R-1-P and catalyzed by uridine phosphorylase (ribosyltransferase uridine: orthophosphate (EC 2.4.2.3) followed by phosphorylation of uridine to 5'-UMP in presence of ATP catalyzed by uridine kinase (5'-phosphotransferase ATP: uridine EC 2.7.1.f) as described on material of animal origin (Canellakis 1957; Sköld 1960; Pontis et al. 1961). The other one would consist on the direct ribotidation of uracil to 5'-UMP in presence of PRPP catalyzed by uracil transphosphoribosylase (phosphoribosyltransferase uridine-5'-monophosphate: pyrophosphate EC 2.4.2.9) as previously described in the case of microorganisms (Crawford et al. 1957).

In order to confirm the operation of these 2 alternative paths of uracil anabolism in higher plants, the attempt was made to detect activities of the 3 named enzymes in experiments in vitro. Previously we have already reported about high uridine kinase activity and considerable catabolic activity of uridine phosphorylase in plant homogenates (Wasilewska and Reifer 1967).

In this communication we report about the presence of these two enzymes in plant extracts, as well as about the presence of uracil transphosphoribosylase, which proves that in plant material uracil may be converted to 5'-UMP on 2 independent paths.

MATERIALS and METHODS

Reagents

Radioactive substrates: 2-¹⁴C-uracil, 2-¹⁴C-uridine, U-¹⁴C-5'-UMP: Radiochemical Centre, Amersham, England; U-¹⁴C-uridine: Prague, Czechoslovakia.

Other reagents: PRPP di-magnesium salt, 2-mercaptoethanol: Koch-light, Colnbrook, England; R-1-P cyclohexylammonium salt: Calbiochem, Los Angeles, USA; ATP disodium salt: Sigma, St. Louis, USA; uracil, uridine, 5'-UMP disodium salt: The Nutritional Bioch. Corp. Ohio, USA; Tris: Loba-Chemie, Vienna, Austria; Glutathion-SH: Schuchardt, Munich, Germany. Other reagents were of Polish origin: Biuro Obrotu Odczynnikami, Gliwice.

Plant material: four species of plants belonging to two families were investigated: green pea (*Pisum sativum*), *Lathyrus tingitanus*, wheat (*Triticum vulgare*) and barley (*Hordeum sativum*). Seeds were soaked for 24 hours in water, sown on trays, covered with moistened tissue paper and permitted to grow under standard conditions for 6–14 days. Extracts were prepared from young plants, cut off 1 cm above the seeds. Acetone powder extracts, supernatant fluids from homogenates and extracts from sulphate of ammonia precipitates were investigated.

Preparation of plant extracts

a) Acetone was powder prepared in the cold by standard methods, using per 100 g of fresh plants 200–300 ml of acetone. The dry powder was kept in a dessicator under a drying agent at temperatures below 0°. 1 g of powder was extracted for 5 minutes at 5° with 30 ml of 0.1 M Tris buffer, pH 7.4 and centrifuged at 9 000 g for 10 minutes.

b) Supernatant fluid was prepared from plants, homogenated in a blender with 3 volumes (pea, *Lathyrus*) or 5 volumes (wheat, barley) of cold 0.1 M phosphate buffer pH 7.2, or Tris-HCl buffer, pH 7.4, containing in each case 5×10^{-4} M of 2-mercaptoethanol. The homogenate was filtered through a double layer of cheese cloth, squeezed out by hand and then centrifuged off for 15 minutes at 13 200 g.

c) Extracts from ammonium sulphate precipitates. The above supernatant fluid was precipitated to 30 % saturation with sulphate of ammonia adding successively small portions of the salt with constant stirring. After 2 hours of standing, the precipitate was centrifuged off as above. To the remaining solution ammonium sulphate was added to 45 % saturation and treated as above. Finally the supernatant was saturated with sulphate of ammonia to 60 %, the precipitate collected after centrifugation and the supernatant discarded. In some cases only 2 protein fractions were prepared i.e. 0–30 % and 30–60 % saturation (wheat), or 0–45 % and 45–60 % saturation (*Lathyrus*). Aliquots of the precipitate were dissolved in 0.1 M Tris-HCl buffer, pH 7.4, containing 10^{-2} M MgCl_2 and 3×10^{-3} M glutathion-SH and filtered through a thin layer of cotton wool. All the extracts were prepared in the cool room at about 5°.

Incubation mixtures:

a) With extracts from acetone powder: to 3 or 6 ml of the extract (about 5 mg protein/ml) were added 0.05–0.5 $\mu\text{mole/sample}$ of ^{14}C -uracil or ^{14}C -uridine, 0.1–1.5 $\mu\text{mole/sample}$ of PRPP, R-1-P or ATP respectively. Total volume of incubate amounted to 3.5 or 6.5 ml.

b) With supernatant fluid: to 2 ml of the supernatant (3–7 mg protein/ml) were added 2.5 $\mu\text{mole/sample}$ of ^{14}C -uracil or ^{14}C -uridine, 3.0 μmoles of PRPP, R-1-P or ATP respectively. Total volume of incubate: 2.5 ml.

c) With ammonium sulphate fractions: to 200 μl of the solution, containing from 1.5–3.5 mg protein were added 0.5 μmole of ^{14}C -uracil or ^{14}C -uridine, 0.6 μmole of PRPP, R-1-P or ATP respectively. Total volume amounted to 300 μl .

The enzymatic reactions were checked against 2 types of controls: 1) the incubation mixture was deprived of PRPP, R-1-P or ATP respectively and 2) the

complete incubation mixture was inactivated at zero time by addition of HClO_4 to a final concentration of 0.3N of the acid. No anabolic reactions were observed in this second control, as no radioactivity was detected in any of the pyrimidine derivatives.

Incubation was carried out at 37° usually for 1 hour. When required the incubation lasted from 15 minutes to 2 hours. The enzymatic reaction was stopped by addition of 3 N HClO_4 to a final concentration of the acid on 0.3 N in the final volume.

Analytical procedure

The radioactive products of the investigated reactions were isolated from the incubation mixture after addition of non-radioactive carriers — uracil, uridine and 5'-UMP- in amounts of 0.5 $\mu\text{mole/sample}$. The analytical procedure for the separation of the 3 uridylic compounds was identical with the one already described (Wasilewska and Reifer 1967), with the omission of ion exchange chromatography on Dowex $1 \times 1 \text{ Cl}^-$. Results were expressed per 1 mg of protein.

Protein was determined according to the method of Lowry et al (1951). Organic phosphate was estimated from the difference between total-P (after combustion) and inorg-P and determined by the method of Fiske and Subba-Row (1925).

DISCUSSION and RESULTS

Results in tables 1, 2 and 3 contain representative data obtained in many experiments with extracts from acetone powders. It is evident from table 1 that in acetone powder extracts from young pea plants, activities of all the 3 enzyme could be demonstrated. Under our experimental conditions uridine kinase has shown highest activities, followed by uridine phosphorylase and uracil transphosphoribosylase, showing considerably lower activity than the first two.

Table 1

Anabolism of uracil and uridine in acetone powder extracts
(10-day old pea plants)

Extract (ml)	^{14}C -Substrate ($\mu\text{mole/sample}$)	Added ($\mu\text{mole/sample}$)	Radioactivity of products (in counts/min/mg of protein)	
			Uridine	5'-UMP
6	2- ^{14}C -Uracil (0.05)	—	4	3
"	"	PRPP(0.2)	8	17
"	"	R-1-P(0.2)	55	9
"	2- ^{14}C -Uridine(0.05)	—	—	26
"	"	ATP(0.2)	—	213

Spec. act. of 2- ^{14}C -uracil and 2- ^{14}C -uridine- 6×10 counts/min/ μmole .
Time of incubation — 2 hours.

It was thought that the lower uracil transphosphoribosylase activity may have been due to the very low concentration of ^{14}C -uracil employed (less than 0.01 mM). Also PRPP which is very unstable in solution could have been the limiting factor. Therefore the concentration of ^{14}C -uracil was increased 10-fold and 3 various concentrations of PRPP were employed, the highest being 5 times in excess over

Table 2

Influence of PRPP and R-1-P concentration on uracil anabolism in acetone powder extracts (6-day old pea plants)

Extract (ml)	^{14}C -Substrate ($\mu\text{mole/sample}$)	Added ($\mu\text{mole/sample}$)	Radioactivity of products (in counts/min/mg of protein)	
			Uridine	5'-UMP
3	2- ^{14}C -Uracil (0.3)	—	6	8
"	"	PRPP (1.5)	695	30
"	"	PRPP (0.5)	334	27
"	"	PRPP (0.1)	205	20
"	"	R-1-P (1.5)	310	8
"	"	R-1-P (0.5)	265	11
"	"	R-1-P (0.1)	268	11

Spec. act. of 2- ^{14}C ;;C-uracil— 9×10^5 counts/min/ μmole . Time of incubation — 1 hour.

the amount of ^{14}C -uracil used (Table 2). As can be seen, excess of PRPP has stimulated considerably the anabolism of uracil, while excess of R-1-P has shown no effect. However it must be pointed out, that incomparably more radioactivity in the experiments with PRPP was detected in uridine and not as was to be expected in 5'-UMP, which alone can be the product of ribotidation of uracil. This observation

Table 3

Influence of time incubation on uracil anabolism in acetone powder extracts (6-day old pea plants)

Extract (ml)	^{14}C -Substrate ($\mu\text{mole/sample}$)	Added ($\mu\text{mole/sample}$)	Time of incub. (min)	Radioactivity of products (in counts/min/mg of protein)	
				Uridine	5'-UMP
3	2- ^{14}C -Uracil(0.5)	U- ^{14}C -5'-UMP(0.004)	120	44850*)	2300*)
"	"	PRPP (0.6)	15	648	54
"	"	"	60	1475	148
"	"	"	120	364	33
"	"	R-1-P (0.6)	15	87	2
"	"	"	60	228	7
"	"	"	120	237	7

Spec. act. of 2- ^{14}C ;;C-uracil— 9×10^5 counts/min/ μmole , U- ^{14}C -5'-UMP— 3×10^5 counts/min/ μmole .

*) In this sample radioactivity of uridine and 5'-UMP is expressed in counts/min/sample.

could only be explained by a process of rapid dephosphorylation of 5'-UMP, owing to the presence in plant material of a very active acid phosphatase.

In order to confirm this contention U- ^{14}C -5'-UMP has been added to the incubation mixture (Table 3). After 2 hours of incubation about 30 % of the label

Table 4

Metabolism of uracil and uridine in ammonium sulphate fractions of green pea
(14-day old plants homogenated in phosphate buffer pH 7.2)

No	Fraction (buffer pH)	¹⁴ C-Substrate	Added	Radioactivity of products (in counts/min/mg of protein)		
				Uracil	Uridine	5'-UMP
I	Supernatant (phosphate 7.2)	2- ¹⁴ C-Uracil	—	—	42	40
	"	"	PRPP	—	120	225
	"	"	R-1-P	—	42	32
	"	2- ¹⁴ C-Uridine	—	1698	—	322
	"	"	ATP	1760	—	980
II	0—30%(NH ₄) ₂ SO ₄ (Tris-HCl 7.4)	2- ¹⁴ C-Uracil	—	—	124	207
	"	"	PRPP	—	9480	14870
	"	"	R-1-P	—	160	280
	"	2- ¹⁴ C-Uridine	—	4160	—	2360
	"	"	ATP	4412	—	18640
III	30—45%(NH ₄) ₂ SO ₄ (Tris-HCl 7.4)	2- ¹⁴ C-Uracil	—	—	82	108
	"	"	PRPP	—	5102	1584
	"	"	R-1-P	—	102	204
	"	2- ¹⁴ C-Uridine	—	6774	—	1592
	"	"	ATP	6920	—	6882
IV	45—60%(NH ₄) ₂ SO ₄ (Tris-HCl 7.4)	2- ¹⁴ C-Uracil	—	—	42	65
	"	"	PRPP	—	210	308
	"	"	R-1-P	—	124	160
	"	2- ¹⁴ C-Uridine	—	5514	—	1160
	"	"	ATP	5760	—	2874

Spec. act. of 2-¹⁴C-uracil and 2-¹⁴C-uridine was 1.8×10^5 counts/min/ μ mole in supernatant fraction and 9×10^5 counts/min/ μ mole in (NH₄)₂SO₄ fractions.

Other details are described in the Material and Methods section.

from radioactive 5'-UMP was detected in uridine, while in the re-isolated 5'-UMP merely 2 % of the introduced radioactivity could be found. This proves conclusively that dephosphorylation of 5'-UMP was the cause of finding over 90 % of the label in the nucleoside under conditions of ribotidation of uracil, which normally (in absence of acid phosphatase) must lead to the formation of the mononucleotide.

Results shown in Tab. 3 also indicate, that extension of time of incubation to 2 hours is undesirable, because no additional activities in the reaction products owing to uridine phosphorylase and uridine kinase catalysis could be observed. Furthermore in the case of uracil transphosphoribosylase a considerable decrease of 5'-UMP has been observed, probably due to thermal inactivation of this enzyme, as heating of a solution of the 0—30 % fraction of ammonium sulphate precipitate for 2 minutes at 61° inactivated the enzyme up to 90 % (unpublished data).

Activities of the 3 enzymes were next investigated in several plants on supernatant fluids from homogenates and various fractions obtained by precipitation of the

Table 5

Metabolism of uracil and uridine in ammonium sulphate fractions of green pea (14-day old plants homogenated in Tris-HCl buffer pH 7.4)

No	Fraction (buffer pH)	¹⁴ C-Substrate	Added	Radioactivity of products (in counts/min/mg of protein)		
				Uracil	Uridine	5'-UMP
I	Supernatant (Tris-HCl 7.4)	2- ¹⁴ C-Uracil	—	—	25	20
	"	"	PRPP	—	114	136
	"	"	R-1-P	—	53	20
	"	U- ¹⁴ C-Uridine	—	760	—	270
	"	"	ATP	667	—	570
II	0—30%(NH ₄) ₂ SO ₄ (Tris-HCl 7.4)	2- ¹⁴ C-Uracil	—	—	136	204
	"	"	PRPP	—	6610	7530
	"	"	R-1-P	—	170	204
	"	U- ¹⁴ C-Uridine	—	2084	—	1084
	"	"	ATP	1998	—	16800
III	30—45%(NH ₄) ₂ SO ₄ (Tris-HCl 7.4)	2- ¹⁴ C-Uracil	PRPP	—	1242	320
	"	"	R-1-P	—	85	64
	"	U- ¹⁴ C-Uridine	ATP	2421	—	7410
IV	45—60%(NH ₄) ₂ SO ₄ (Tris-HCl 7.4)	2- ¹⁴ C-Uracil	PRPP	—	115	86
	"	"	R-1-P	—	86	82
	"	U- ¹⁴ C-Uridine	ATP	2886	—	2280

Spec. act. of 2-¹⁴C-uracil and U-¹⁴C-uridine was 6×10^5 counts/min/ μ mole in supernatant fraction and 3×10^5 counts/min/ μ mole in (NH₄)₂SO₄ fractions.

Other details are described in the Material and Methods section.

active principle with sulphate of ammonia (Tables 4—8). Results shown in table 4 present one of 3 preparations made from young pea plants. Considerable PRPP-dependent uracil anabolism and uridine phosphorylation to 5'-UMP could be observed, but different than in extracts from acetone powder, no conversion of uracil to uridine catalysed by uridine phosphorylase could be detected. However the reverse catabolic effect of the enzyme was confirmed as witnessed by the appearance of radioactive uracil produced from ¹⁴C-uridine.

Fractionation with ammonium sulphate permitted to localise most of uracil transphosphoribosylase activity at 0—30 % saturation. In this fraction also over 50 % of the uridine kinase activity could be detected. The catabolic uridine phosphorylase activity was found in the precipitates of ammonium sulphate fractions.

Two points of interest emerge from the results in table 4: a) no R-1-P-dependent anabolism of uracil could be observed, although the catabolic activity of uridine phosphorylase was very marked and b) there was considerable conversion of uridine to 5'-UMP in samples, where no ATP has been added.

Plant homogenates which were used for ammonium sulphate fractionation for the experiments in table 4, were prepared with phosphate buffer. The fractions

Table 6

Metabolism of uracil and uridine in ammonium sulphate fractions of *Lathyrus* (14-day old plants homogenated in phosphate buffer pH 7.2)

No	Fraction (buffer pH)	¹⁴ C-Substrate	Added	Radioactivity of products (in counts/min/mg of protein)		
				Uracil	Uridine	5'-UMP
I	Supernatant (phosphate 7.2)	2- ¹⁴ C-Uracil	—	—	30	13
	"	"	PRPP	—	67	173
	"	"	R-1-P	—	42	25
	"	2- ¹⁴ C-Uridine	—	828	—	102
	"	"	ATP	732	—	196
II	0—45% (NH ₄) ₂ SO ₄ (Tris-HCl 7.4)	2- ¹⁴ C-Uracil	—	—	169	45
	"	"	PRPP	—	3440	2910
	"	"	R-1-P	—	352	86
	"	2- ¹⁴ C-Uridine	—	3975	—	300
	"	"	ATP	3300	—	1335
III	45—60% (NH ₄) ₂ SO ₄ (Tris-HCl 7.4)	2- ¹⁴ C-Uracil	—	—	40	63
	"	"	PRPP	—	160	190
	"	"	R-1-P	—	38	60
	"	2- ¹⁴ C-Uridine	—	4484	—	205
	"	"	ATP	3630	—	562

Spec. act. of ¹⁴C-substrates as in Tab. 5.

could therefore contain relatively much orthophosphate which is one of the products of the anabolic reaction. As is well known, under these conditions the reverse reaction (uridine → uracil) is favoured (Canellakis 1957). Therefore a pea plant homogenate was prepared with 0.1 M Tris-HCl in place of phosphate buffer. Under these conditions the ammonium sulphate fractions would contain only some of the endogenous phosphates. Thus synthesis of uridine from uracil and R-1-P should have been favoured, rather than deribosidation of the nucleoside. However, results shown in table 5 did not confirm our views, as no uridine synthesis could be detected and catabolism of the nucleoside was confirmed in all fractions.

Some detectable phosphorylation of uridine in experiments, where no ATP has been added may be due to the presence of endogenous high energy phosphate compounds. In the investigated ammonium sulphate fractions organic phosphate was detected (table 5: fraction I — 60, II — 10, III — 9 and fraction IV — 3 µg/sample) which would seem to support the above suggestion.

Results shown in tables 6, 7 and 8 confirm the presence of the 3 enzymes also in other plants i.e. *Lathyrus*, wheat and barley.

In all investigated plants extensive dephosphorylation of 5'-UMP has been observed, owing to the presence of acid phosphatase. Fortunately much of phosphatase activity was found in the ammonium sulphate fractions above 30% saturation, thus in the fraction from 0—30% saturation the activities of uridine

Table 7

Metabolism of uracil and uridine in ammonium sulphate fractions of wheat (7-day old plants homogenated in Tris-HCl buffer pH 7.4)

No.	Fraction (buffer pH)	¹⁴ C-Substrate	Added	Radioactivity of products (in counts/min/mg of protein)		
				Uracil	Uridine	5'-UMP
I	Supernatant (Tris-HCl 7.4)	2- ¹⁴ C-Uracil	—	—	26	23
	"	"	PRPP	—	116	110
	"	"	R-1-P	—	60	20
	"	U- ¹⁴ C-Uridine	—	450	—	126
	"	"	ATP	490	—	280
II	0—30%(NH ₄) ₂ SO ₄ (Tris-HCl 7.4)	2'- ¹⁴ C-Uracil	—	—	90	78
	"	"	PRPP	—	912	1175
	"	"	R-1-P	—	108	78
	"	U- ¹⁴ C-Uridine	—	1125	—	437
	"	"	ATP	1206	—	1218
III	30—60%(NH ₄) ₂ SO ₄ (Tris-HCl 7.4)	2- ¹⁴ C-Uracil	—	—	40	38
	"	"	PRPP	—	468	66
	"	"	R-1-P	—	32	32
	"	U- ¹⁴ C-Uridine	—	2380	—	148
	"	"	ATP	2358	—	450

Spec. act. of ¹⁴C-substrates as in Tab. 5.

kinase and uridine transphosphoribosylase could be satisfactorily detected. Owing to the presence of phosphatase it must be assumed that uridine kinase activity is much higher than would appear from radioactivity actually detected in the re-isolated 5'-UMP. This however could not be directly proved as in the case of 5'-UMP synthesis from uracil and PRPP, because the substrate of phosphorylation as well as the product of dephosphorylation was ¹⁴C-uridine.

Tunis and Chargaff (1960) succeeded in separation of acid phosphatase from nucleoside phosphotransferase from carrot roots, by means of electrophoresis after absorption of phosphatase on Celite. We are now trying to obtain preparations of uracil transphosphoribosylase and uridine kinase free of most of phosphatase activity from the green pea. This plant has shown highest activity of the 2 enzymes and furthermore in acetone powders from the green pea, considerable anabolic activity of uridine phosphorylase could be detected.

It appears from results presented in this paper, that higher plants contain all the three enzymes required for the operation of these two alternative paths of uridine-5'-monophosphate synthesis, as previously postulated from our studies in vivo (Wasilewska and Reifer 1967). Reichard and Sköld (1957) who investigated enzymic formation of uridine monophosphate from uracil in acetone powder extracts from Ehrlich ascites tumor also demonstrated two different mechanisms acting simultaneously involving uridine phosphorylase, followed by uridine

Table 8

Metabolism of uracil and uridine in ammonium sulphate fractions of barley (10-day old plants homogenated in phosphate buffer pH 7.2)

No.	Fraction (buffer pH)	¹⁴ C-Substrate	Added	Radioactivity of products (in counts/min/mg of protein)		
				Uracil	Uridine	5'-UMP
I	Supernatant (phosphate 7.2)	2- ¹⁴ C-Uracil	—	—	46	42
	"	"	PRPP	—	192	158
	"	"	R-1-P	—	82	55
	"	2- ¹⁴ C-Uridine	—	941	—	236
	"	"	ATP	875	—	520
II	0—30% (NH ₄) ₂ SO ₄ (Tris-HCl 7.4)	2- ¹⁴ C-Uracil	—	—	84	48
	"	"	PRPP	—	1018	905
	"	"	R-1-P	—	282	104
	"	2- ¹⁴ C-Uridine	—	1374	—	608
	"	"	ATP	1270	—	2126
III	30—45% (NH ₄) ₂ SO ₄ (Tris-HCl 7.4)	2- ¹⁴ C-Uracil	—	—	48	42
	"	"	PRPP	—	786	78
	"	"	R-1-P	—	130	72
	"	2- ¹⁴ C-Uridine	—	2100	—	348
	"	"	ATP	1916	—	1193
IV	45—60% (NH ₄) ₂ SO ₄ (Tris-HCl 7.4)	2- ¹⁴ C-Uracil	PRPP	—	240	72
	"	"	R-1-P	—	103	48
	"	2- ¹⁴ C-Uridine	ATP	2130	—	834

Spec. act. of ¹⁴C-substrates as in Tab. 5.

kinase, or direct condensation of uracil and PRPP to 5'-UMP catalysed by uracil transphosphoribosylase.

As far as we know, no report has been published previously on the appearance of all the 3 enzymes simultaneously in material obtained from higher plants. Further work on purification of the 3 enzymes and on their kinetic characteristics is in progress.

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SUMMARY

3 enzymes participating in two alternative paths of 5'-UMP synthesis from uracil have been detected in higher plants: uridine phosphorylase (EC 2.4.2.3) which catalyses the ribosidation of uracil to uridine in presence of R-1-P, uridine kinase (EC 2.7.1.f) responsible for uridine phosphorylation to 5'-UMP in presence of ATP and uracil transphosphoribosylase (EC 2.4.2.9) catalysing the direct transfer of the ribosylphosphate residue from PRPP to uracil.

Extracts from 4 plants were investigated i.e. *Pisum sativum*, *Lathyrus tingitanus*, *Triticum vulgare* and *Hordeum sativum*.

Activity of all 3 enzymes were detected in extracts from acetone powder prepared from young pea plants.

Fractionation with ammonium sulphate permitted to localise the bulk of uracil transphosphorylase and uridine kinase activity in the fraction of 0—30% saturation.

Uridine phosphorylase was present in all fractions of the investigated plants, but only the catabolic path (uridine → uracil) could be observed.

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Przemiany uracylu i urydyny in vitro w roślinach wyższych

Streszczenie

W wyciągach z materiału roślinnego wykazano aktywność 3 enzymów biorących udział w syntezie 5'-UMP z uracylu. Są to: fosforylaza urydynowa (EC 2.4.2.3), katalizująca rybozydację uracylu do urydyny w obecności R-1-P; kinaza urydynowa (EC 2.7.1.f), odpowiedzialna za fosforylację urydyny do 5'-UMP kosztem ATP i transfosforybozylaza uracylowa (EC 2.4.2.9), katalizująca bezpośrednie przeniesienie reszty rybofosforanowej z PRPP na uracyl z wytworzeniem 5'-UMP.

Przebadano 4 rośliny: groch (*Pisum sativum*), łędwian (*Lathyrus tingitatus*), pszenicę (*Triticum vulgare*) i jęczmień (*Hordeum sativum*).

Aktywność enzymów badano w ekstraktach z proszków acetonowych (groch) w supernatantach homogenatów i wyciągach białkowych osadów z siarczanu amonu (groch, łędwian, pszenica, jęczmień).

Wykryto aktywność anaboliczną wszystkich 3 enzymów w ekstraktach z proszku acetonowego grochu.

Fracjonowanie siarczanem amonu pozwoliło na zlokalizowanie większości aktywności transfosforybozylazy uracylowej i kinazy urydynowej we frakcji 0—30% nasycenia $(\text{NH}_4)_2\text{SO}_4$.

Fosforylaza urydynowa obecna była we wszystkich frakcjach. W tych frakcjach zdolano jednak stwierdzić jedynie jej katabolizny kierunek działania.