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The effect of salinity in the growth medium carbohydrate metabolism in pea root tips

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The effect of chloride and sulphate types of salinity on respiratory mechanism of pea roots was studied. Both types of salinity depressed the absorption of external glucose and the rate of respiration and changed the relative part of the metabolic pathways. The percentage of the pentose-phosphate pathway was increased by increasing levels of NaCl salinity while Na_2SO_4 salinity practically did not affect it. Chloride salinity depressed CO_2 evolution from C-6 but did not affect the CO_2 evolution from C-1. Sulphate salinity depressed both. Most of the glycolytic enzymes studied were depressed in roots grown in saline medium except glucose-phosphate isomerase which was not affected by sulphate salinity but was increased more than ten fold by chloride salinity. Phosphogluconate dehydrogenase was not affected by salinity. Glucose-6-phosphate dehydrogenase was found in the mitochondria as well as in the supernatant and both the mitochondrial and the supernatant enzymes were active with NADP and NAD. Salinity of both types increased the NADP linked activity in the soluble fraction. The information gained however is still not sufficient to explain adequately the effect of salinity on plant metabolism.

In a previous paper (*1*) it was reported that both chloride and sulphate types of salinity affect carbohydrate metabolism in pea root tips. Although the effect of the two types of salinity was not strictly identical, the general trend was that the C-6/C-1 ratio decreased with increasing salinity. The method used in the previous study was open to the criticism raised by KATZ and WOOD (*2, 3, 4*) since the C-6/C-1 ratio was computed from the yield of CO_2 calculated as percentage of the total radioactivity in the Warburg flask.

The whole investigation was, therefore, repeated and the yield of CO_2 was calculated as percentage of radioactive glucose absorbed by the tissue. Moreover, the investigation was extended and the effect of salinity on the activity of some of the glycolytic enzymes was studied. In addition,

the studies of the effect of salinity on the activity of glucose-6-phosphate dehydrogenase (*1*) and phosphogluconate dehydrogenase were extended.

Materials and methods

Pea seeds (*Pisum sativum*, variety Laxton Progress) were used in these experiments. The seeds were imbibed in water for three hours and then germinated in vermiculite moistened with HOAGLAND solution, or HOAGLAND salinized with NaCl or Na_2SO_4 to the desired concentration. One atmosphere of osmotic potential was considered to be produced by 24 meq. NaCl or by 34 meq. of Na_2SO_4, per litre. The osmotic potential of the non-salinized HOAGLAND was equivalent to 0.5 atmosphere, however this is not included in the concentrations given in the various tables. The plants were grown in a constant temperature room, at 26° and with continuous illumination.

One cm long root tips, in lots of 100 mg, were incubated in shaking Warburg flasks, at 26° during 3 hours. The flasks contained glucose, labelled either at C-1, C-6 or C-U, dissolved in the same medium in which the roots were grown. The activity in each flask was 0.025 μc/ml. The labelled glucose was purchased from the Radiochemical Centre, Amersham, England.

The absorption of glucose was computed from the difference in radioactivity of the external medium at the beginning and the end of the experiment. This was checked by few combustion experiments (*5*). As no difference was found, the former calculation was used.

The CO_2 liberated in respiration was collected in KOH. The radioactivity of all samples was measured in a Tricarb-Packard scintillation counter using PPO (2,5-diphenyloxazol) and POPOP (1,4-bis-(5-phenyloxazol-2-yl)benzene) in toluene, methanol and ethanol amine (*6*).

For measurements of enzymic activity 1 g lots of roots, 1 cm long, were collected from the main and lateral roots and homogenized with 10 ml of phosphate-sucrose buffer, pH 7.9. The buffer contained 0.1 M phosphate, 0.4 M sucrose, 10^{-3} M $MgSO_4 \cdot 7H_2O$ and 1 ppm EDTA. The homogenate was strained through cheese cloth and centrifuged at $500 \times g$. A mitochondrial fraction was sedimented from the resulting solution, at $20{,}000 \times g$. The resuspended mitochondrial fraction and the supernatant were used for assays of glucose-6-phosphate dehydrogenase. For all other enzymes the supernatant after centrifugation at $100{,}000 \times g$ was used.

Glucosephosphate isomerase activity (EC 5. 3. 1. 9) was measured according to COOPER et al. (*7*), fructokinase (EC 2. 7. 1. 4) was measured according to WU and RACKER (*8*), triosephosphate isomerase (EC 5. 3. 1. 1) according to BIEZENHERZ (*9*) and pyruvate kinase (EC 2. 7. 1. 40) according to BUCHER and PFLEIDERER (*10*). Glucose-6-phosphate dehydrogenase (EC 1. 1. 1. 49) was measured as previously described (*1*) and phosphogluconate dehydrogenase (EC 1. 1. 1. 44) was measured according to HORECKER and SMYRNIOTIS (*11*).

Protein was estimated according to LOWRY et al. (*12*).

Results

Glucose absorption by pea roots grown at different levels of salinity was measured. CO_2 evolved from C-6, C-1 or uniformly labelled glucose was collected and its activity calculated as a percentage of the activity absorbed. From these results C-6/C-1 ratios were computed (Table 1).

As can be seen from Table 1, increased levels of salinization with NaCl resulted in an increased depression of glucose absorption, reaching nearly 40% at 5 atmospheres. When the medium was salinized with Na_2SO_4, glucose absorption was practically unaffected until salinity rose to the level of 5 atm. At this level of salinity absorption was depressed by almost 50 %.

Liberation of CO_2 from C-U glucose increased at the higher levels of

Table 1
The effect of salinity on sugar absorption, CO_2 evolved and C-6/C-1 ratio in pea root tips

Salinity (atm)	Chloride salinity					Sulphate salinity				
	% Sugar absorbed	CO_2 (C-U)	CO_2 (C-6)	CO_2 (C-1)	C-6/C-1	% Sugar absorbed	CO_2 (C-U)	CO_2 (C-6)	CO_2 (C-1)	C-6/C-1
0.0	49	22	34	44	0.77	49	22	34	44	0.77
1.0	39	22	28	45	0.63	48	23	18	36	0.50
3.0	36	29	26	43	0.60	45	30	15	32	0.47
5.0	31	30	21	44	0.49	27	31	13	30	0.43

The plants were grown in medium salinized either with NaCl or Na_2SO_4.
Sugar absorption was calculated as percentage of the external sugar and labelled CO_2 as percentage of the absorbed activity. Zero salinity refers to non-salinized HOAGLAND solution.

Table 2
Percentage of glucose metabolized by pentosephosphate pathway under different conditions of salinity

Salinity (atm)	Percent pentosephosphate pathway	
	Chloride	Sulphate
0	6	6
1	9	8
3	10	7.5
5	12	7.5

Calculation was made according to the following equation proposed by WOOD and KATZ (*13*):

$$\frac{CO_2(G_1)-CO_2(G_6)}{1-CO_2(G_6)}=\frac{3Pc}{1+2Pc}$$

$CO_2(G_1)$ and $CO_2(G_6)$ are the specific yields of CO_2 liberated from glucose labelled either at C-1 or C-6.

salinity. No difference was observed between the effects of chloride and sulphate. Liberation of CO_2 from C-1 was not affected in pea roots grown under chloride salinity. However, it was depressed with increasing sodium sulphate concentration. Liberation of CO_2 from C-6 was depressed with the increasing salinity of both types. Sulphate caused a much more severe and abrupt effect. As a result of these changes C-6/C-1 ratio decreased to a greater extent in pea roots grown under sulphate conditions.

Since the EMBDEN-MEYERHOF and pentosephosphate pathways seem to be the only routes of carbohydrate oxidation in plants, it is legitimate to calculate the pentosephosphate value for each of the treatments; The results are given in Table 2.

The specific activity of the various glycolytic enzymes studied is given in Tables 3, 4, 5 and 6. As can be seen from these results, sulphate and chloride types of salinity depressed the specific activities of fructokinase, triosephosphate isomerase and pyruvate kinase in a similar manner. With some enzymes chloride salinity was more effective, in others sulphate

Table 3

Effect of salinization of growth medium with NaCl on specific activity of some glycolytic enzymes in pea root tips

Enzyme	Salinity (atm)			
	0.0	1.0	3.0	5.0
Glucosephosphate isomerase	0.04	0.10	0.30	0.60
Fructokinase	0.18	0.11	0.11	0.12
Triosephosphate isomerase	0.20	0.16	0.15	0.12
Pyruvate kinase	0.27	0.13	0.12	0.12

Results are given as units O.D./mg protein/min.
The results are means of at least three different experiments each of them in duplicate.

Table 4

Effect of salinization of growth medium with Na_2SO_4 on specific activity of some glycolytic enzymes in pea root tips

Enzyme	Salinity (atm)			
	0.0	1.0	3.0	5.0
Glucosephosphate isomerase	0.04	0.03	0.03	0.04
Fructokinase	0.18	0.13	0.14	0.14
Triosephosphate isomerase	0.20	0.13	0.11	0.07
Pyruvate kinase	0.27	0.21	0.09	0.06

Results are given as units O.D./mg protein/min, salinity is expressed in atmospheres.
The results are means of at least three different experiments each of them in duplicate.

salinity was more effective. However, the effect of the two types of salinity on the specific activity of glucosephosphate isomerase was completely different: In roots grown under sulphate salinity the specific activity of this enzyme was practically unaffected by increasing level of salinity and always remained very low. In roots grown under chloride salinity the specific activity of this enzyme rose considerably with increasing level of salinity, up to 15 times the value in the non-saline controls.

From the enzymes active in the pentose phosphate pathway, glucose-6-phosphate dehydrogenase and phosphogluconate dehydrogenase were studied. Their specific activities are given in Tables 5 and 6. The activity of phosphogluconate dehydrogenase was not affected by salinity.

Glucose-6-phosphate dehydrogenase was shown to be present in the mitochondrial fraction and in the supernatant. It was also shown to be active with both NADP and NAD (Table 6). As can be seen from Table 6, in roots grown under chloride salinity the activity of the enzymes

Table 5

Effect of salinity on specific activity of phosphogluconate dehydrogenase in pea roots grown in different types of salinity

	Chloride salinity (atm)				Sulphate salinity (atm)			
	0.0	1.0	3.0	5.0	0.0	1.0	3.0	5.0
Phosphogluconate dehydrogenase	0.05	0.04	0.05	0.05	0.05	0.05	0.05	0.05

Salinity was induced either by NaCl or by Na_2SO_4. Specific activity is given as units O.D./mg protein/min.
The results are means of at least three different experiments.

Table 6

Effect of salinity on specific activity of mitochondrial and soluble glucose-6-phosphate dehydrogenase

Fraction	Salinity (atm)	Salinity-type			
		Chloride		Sulphate	
		with NADP	with NAD	with NADP	with NAD
Mitochondrial	0	1.30	1.31	1.30	1.31
	1	1.31	1.20	0.74	1.22
	3	1.05	1.00	1.66	2.20
	5	0.83	0.82	1.03	1.55
Soluble	0	0.30	1.11	0.30	1.11
	1	0.76	0.42	0.37	0.44
	3	0.80	0.41	0.46	0.49
	5	0.83	0.25	0.46	0.77

Mitochondria were spun down at 20,000 × *g*.
Results as units O.D./mg protein/min, salinity in atmospheres.

linked to NADP in the mitochondria decreases while the soluble one increases with increasing salinity. The NAD linked activity decreases with increasing salinity in both, mitochondrial and soluble fractions.

The situation is not so clear for the enzymes isolated from root tips grown in Na_2SO_4. There is a definite effect of salinity on the soluble enzyme and it is very similar to the effect of chloride salinity but less pronounced. The mitochondrial enzyme behaves very irregularly.

Discussion

The experiments described above show clearly that presence of salinity in the growth medium affects the respiratory mechanism of the plant roots. The effect is brought about in a number of ways: The capacity of the tissue to absorb exogenous glucose is lowered. Respiration in the presence and absence of exogenous glucose is depressed. The relative importance of the EMBDEN-MEYERHOF and pentosephosphate pathways is changed.

Both types of salinity reduce glucose absorption by the root tissue (Table 1). Under chloride salinity the reduction is gradual with increasing salinity. Under sulphate salinity there is almost no change in glucose absorption until the salinity reaches 5 atm, at which level the absorption is reduced abruptly by almost 50 %.

Contrary to the findings of LIVNE and LEVIN (*14*) the respiration of the root tips, as measured by oxygen consumption (although not reported in this paper) was lower, the higher was substrate salinity. This was noted both in the presence and in the absence of exogenous glucose. However, $^{14}CO_2$ evolution during respiration of uniformly labelled exogenous glucose absorbed, increased with increasing salinity (Table 1), while the absolute amount of $^{14}CO_2$ output did not change significantly.

The percentage of the pentosephosphate pathway of the total respiration as calculated from the C-6 and C-1 yields according to WOOD and KATZ (*13*) is given in Table 2. From these results it is clear that with increasing levels of chloride salinity in the mdium, the relative importance of the pentose phosphate cycle increases. As can be seen from Table 1 the specific yield of CO_2 from C-6 decreases, while that from C-1 is not affected. Under sulphate salinity the percentage of pentosephosphate pathway remains more or less constant at the various levels of salinity (Table 2). However, the specific yield of CO_2 from both C-1 and C-6 decreases with increasing salinity (Table 1). The changes in C-6/C-1 ratio in the two types of salinity therefore reflect different ways of affecting respiratory metabolism. Changes in respiration, a decrease in glycolysis and an increase in the pentosephosphate pathway have been shown to exist in wheat plants grown under water stress (*15*). Similar changes are also frequently observed in plant tissues in response to adverse conditions

such as fungal infection, herbicide treatment, slicing and as a result of ageing (*16*, *17*, *18*, *19*, etc.).

In order to understand some of these changes better, a study of several enzyme systems participating in the two alternate metabolic pathways was undertaken (Tables 3, 4, 5 and 6). The activity of most of the glycolytic enzymes studied was depressed by increasing levels of salinity of both types. Fructokinase is often considered as a key enzyme determining the rate of glycolysis (*20*, *21*, *22*). In heart muscle the reduction of fructokinase activity in cation rich media resulted in a depression of glycolysis (*21*). But the effect was considered to be indirect, through a lowering of the level of ADP and inorganic phosphate. The results in Tables 3 and 4 show that due to salinity the activity of this enzyme is reduced nearly by 30 %. This is an indication for a possible direct effect of the absorbed salts on the enzyme. As a result, glycolysis may be depressed.

The activity of pyruvate kinase and triosephosphate isomerase is also lower in roots grown in saline media than in the controls (Tables 3 and 4). The inhibition caused by sulphate salinity is greater than that caused by chloride salinity. These results agree with those of other workers (*23*) who showed greater inhibition of triosephosphate isomerase by sulphate ions than by chloride ions. The lowered activity of these two enzymes may also have a role in repressing glycolysis (*22*).

A very pronounced difference in the effect of the two types of salinity on glucosephosphate isomerase was observed (Tables 2 and 3). While sulphate salinity did not affect this enzyme at all, under chloride salinity its activity was increased more than ten times. However, the role of this enzyme in controlling the rate of conversion of glucose to pyruvate is still in dispute (*24*, *25*, *26*). Phosphogluconate and glucose-6-phosphate dehydrogenases were affected differently by salinity. The former was hardly affected at all, while the activity of the latter (NADP linked enzyme in soluble fractions) increased in salt treated plants, chloride being more effective than sulphate. Assuming that this enzyme is rate limiting, this increase in activity can, at least partly, explain the increased contribution of the pentosephosphate pathway in respiration. A similar differential effect on the two enzymes was found in rat liver due to treatment with ethionine (*27*, *28*). It must be noted that the different effects of salinity on the different types of glucose-6-phosphate dehydrogenase, i. e. on those functioning with NAD and NADP and on the soluble and mitochondrial enzymes, cannot at present be interpreted satisfactorily. Such different enzymes have been reported in other tissue also (*29*, *30*), but there too their relative rates and importance is still unclear. It is worthwhile noting that the NAD linked activity under chloride salinity decreases both in the mitochondrial and in soluble fractions, while the NADP linked activity markedly decreases in mitochondria and increases in the supernatant. Sulphate salinity has the same effect on the soluble enzyme, while the effect on the mitochondrial enzyme

is not clear-cut.

The results we have brought so far indicate the profound and complicated changes induced by salinity in respiratory mechanism. They also indicate differences due to sulphate and chloride salination. However, a clear interpretation of the effect of salinity is not yet possible and much more extensive and detailed studies are still necessary. It seems that the effect of salinity on plant metabolism is rather complicated and probably is a combination of osmolarity, ionic strength and the specific effect of the ions.

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