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Effect of Substrate Salinity on the Ability for Protein Synthesis in Pea Roots[1]

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Abstract. The effect of salinity on incorporation of amino acids into root tip protein is apparently of dual nature: in presence of salts the uptake is depressed and the normal metabolic pathways are disturbed. If the roots were grown at high salt concentration, uptake and incorporation are affected even if they are carried out in the absence of salt. NaCl and Na_2SO_4 affect uptake, incorporation, and metabolism of ^{14}C leucine in different ways. There are also preliminary indications that in pea roots grown at different types of salinity, different proteins may be synthesized. Kinetin was found to inhibit incorporation of amino acids into non stressed and Na_2SO_4 stressed roots, but promotes uptake and incorporation of amino acids into protein in NaCl stressed tissue. It seems that there are some pronounced differences between the effects of NaCl and Na_2SO_4 salinities on the metabolism of pea root tissue.

The effect of salinity on various aspects of plant metabolism is intensively investigated at present (1, 7, 10, 11, 12). Some attempts have been made to compare the effect of salinities induced by NaCl and Na_2SO_4. Carbohydrate metabolism was shown to be affected differently by these 2 types of salinity (10, 11). Other aspects of metabolism have also been shown to be sensitive to salinity, mainly of the NaCl type (1, 7, 12). Reversal, to some extent, by kinetin of the deleterious effects of NaCl was shown (1, 4).

In the work reported here an attempt was made to study the effects of NaCl and Na_2SO_4 salinities on protein synthesis in pea roots. In preliminary experiments the ability of kinetin to reverse these effects was investigated.

Materials and Methods

Root tips of garden pea plants (*Pisum sativum*, var. Laxton Progress) were used in these experiments. The seeds were surface sterilized by immersion for 10 minutes in a solution of commercial sodium hypochlorite (diluted 1:20), and then rinsed in running water for 10 minutes. Lots of 35 seeds were germinated in No. 4 vermiculite in round plastic containers of 1.5 kg capacity. The vermiculite was saturated with Hoagland's solution (2) or Hoagland salinized with either NaCl or Na_2SO_4. All containers were adjusted at the beginning of the experiment to a constant initial weight. Water loss (less than 2 % of the solution in the container) was replenished daily. The water was added alternatively from the top and from the bottom (through a central glass tube) to avoid as much as possible the formation of salinity gradient in the container. The containers were kept in a constant conditions growth chamber (20° and 60–80 % R.H.) with continuous illumination of 200 to 300 ft-c at the top of the container. Roots from 10 days old seedlings were collected, and washed in a large volume of sterile medium identical in composition to that of the growth medium. All further work was carried out under sterile conditions.

Incorporation Studies. Fifteen root tips, 5 mm long, were preincubated in glass vials in tris-maleate (T-M) buffer 0.05 M (pH 5.0) containing 10^{-3} M Mg^{2+}; 5×10^{-4} Ca^{2+}; NaCl or Na_2SO_4 according to the desired salinity and chloramphenicol, streptomycin, and penicillin G, 50 μg per ml, each. The roots were shaken in the above medium (80 strokes/min) during 30 minutes at 25°. Then labeled amino acid (1-leucine ^{14}C), dissolved in the same buffer was added. The final volume of the reaction mixture was 1 ml. Incorporation was allowed to proceed for 2 hours, under the same conditions as preincubation. At the end of the 2 hours the reaction was stopped by immediate cooling of the vials in crushed ice. The root segments were collected on sintered glass discs, on a millipore filter holder, and rinsed with cold T-M buffer containing 5 mg/ml unlabeled L-leucine (T-M-L). They were then immersed for a further 10 minutes in the same T-M-L buffer. The root tips were homogenized in 5 ml T-M-L buffer. Two samples were taken from the homogenate for incorporation measurements and 1 sample for protein estimation according to Lowry *et al.* (6).

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Amino acid incorporation into the trichloroacetic acid precipitates was determined. These were prepared from the homogenates with 10 % trichloroacetic acid containing unlabeled leucine (10 mg/ml). The precipitates were cooled, collected, and resuspended in 5 % containing leucine, then heated at 90° for 20 minutes. The precipitates were finally collected on a Matheson Higgins Microfilter (0.45 μ pore size). The filter disc was dried and placed into scintillation fluid (4 g PPO + 100 mg POPOP/liter toluene) and counted in 3002 model Packard Counter, at 80 % efficiency.

Results of different experiments were corrected for 80 % efficiency. The total weight of precipitate on a filter disc did not exceed 1 mg. Protein content of such a sample was usually around 200 μg.

Controls for incorporation of labeled amino acid by microorganisms present in reaction mixture were run on incubation medium from which the roots were removed after preincubation and prior to addition of the labeled amino acid. Precipitates of these controls were prepared and treated as described above.

Balance of ^{14}C From L-*leucine That Entered the Root.* The experiment was carried out in Warburg flasks, under the same conditions as described above. Twenty-three root tips, 5 mm long were preincubated for 30 minutes in 1.6 ml T-M-L buffer. At the end of this time the buffer was removed and 1.6 ml of the same buffer containing 0.5 μc/ml L-leucine ^{14}C (275 mc/mmole) was added. The centre well of the flask contained 0.2 ml KOH 10 % and a fluted filter paper. The side arm of the flask contained 0.1 ml 5 M H_2SO_4.

Respiration was measured at 25°. After 2 hours the acid from the side arm was poured in, and the flasks were shaken for a further 10 minutes and then put into crushed ice. The $K_2{}^{14}CO_3$ on the filter paper was counted in a scintillation fluid containing 10 ml of counting fluid described above plus 8.9 ml methanol plus 1 ml ethanolamine. This gave the ^{14}C liberated as CO_2. The root tips were rinsed as described above then divided into 3 lots: 2 lots, 9 roots each, were used for combustion according to Kelly *et al.* (5) as modified by Harel (3) and the third lot of 5 roots was homogenized and the incorporation of leucine into protein was measured as described above. All experiments of incorporation were carried out between 9:00 and 12:00 AM, to avoid any differences due to a diurnal cycle.

Leucine-^{14}C was purchased either from Radiochemical Centre, Amersham, England, or New England Nuclear Corporation, Massachusetts. Amino acid mixture was purchased from New England Nuclear Corporation. All other chemicals were of analar or scintillation grade.

The osmotic potential of Hoagland's solution was 0.8 atm, but it is not taken into account in the tables and in the graphs. For salinization. 1.4 g/l NaCl or 2.27 g/l Na_2SO_4 were used for creating an osmotic potential of 1 atmosphere.

Results

The incorporation of L-leucine ^{14}C into protein from solutions having various activities (0.25–1.0 μc/ml) was studied. The reaction mixture had the same level and composition of salinity as the growth medium. The results are given in figure 1. It is evident from these results that the higher the salinity in the medium (in this case growth medium and reaction mixture), the lower is the incorporation of the amino acid. Moreover, sulfate salinity is more inhibiting than chloride salinity. In the low range of salinity (control, 1 atm and 3 atm chloride), at external activities higher than 0.5 mc per flask, no appreciable increase in incorporation could be observed. In the higher range of salinity (5 and 7 atm NaCl and 3, 5, and 7 atm sulphate), the incorporation seems to increase linearly with increasing activity. Nevertheless, in most of the following experiments 0.5 μcurie per flask were used.

The results shown in figure 1 do not enable to differentiate between the effect of salinity during growth, on the ability of the roots to incorporate the amino acid and between the interference of salt, present in the reaction mixture, with absorption and incorporation of the amino acid. The incorporation of labeled leucine was therefore studied in roots grown in presence of salt and incorporating in its absence, as well as in roots grown in absence of salts and incorporating in its presence; the results

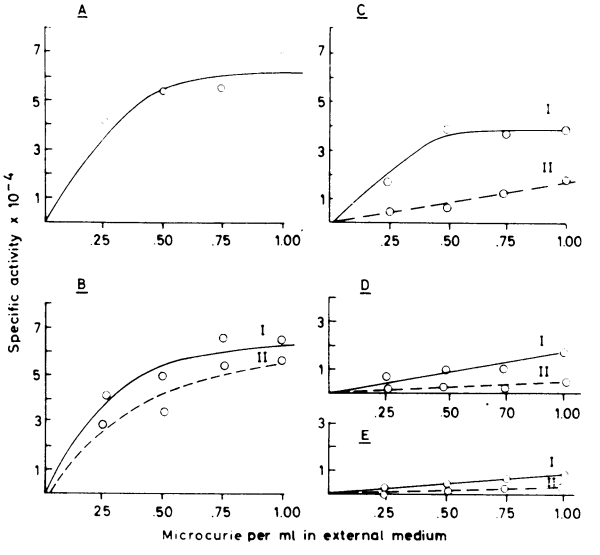

FIG. 1. Relationship between activity in external medium and incorporation of l-leucine-^{14}C. Incorporation experiments were carried out in medium of the same composition as the growth medium. Specific activity of leucine was 6.6 mc/mmole. I—chloride salinity, II—sulfate salinity, A—control, B—medium salinized to 1 atm, C—medium salinized to 3 atm, D—medium salinized to 5 atm, E—medium salinized to 7 atm. The results are expressed as specific activity of the TCA insoluble precipitate, *i.e.* cpm/mg protein.

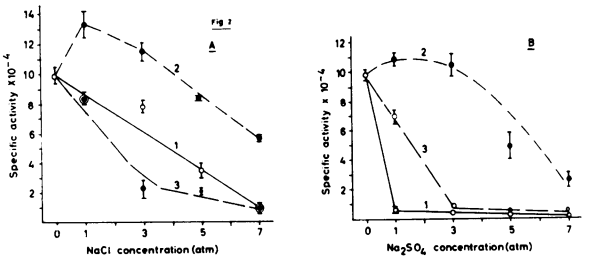

FIG. 2. The effect of various types and various levels of salinity on incorporation of l-leucine-^{14}C into pea root tips. l-leucine-^{14}C-(U) was used in the experiments. The specific activity of the leucine was 6.6 mc/mmole. 0.5 μcurie per ml were used. Results expressed as in figure 1. A—chloride salinity, B—sulfate salinity. 1. Plants grown in saline medium, incorporation measured in the same saline medium. 2. Plants grown in saline medium, incorporation measured in non-saline medium. 3. Plants grown in non-saline medium, incorporation measured in saline medium.

are given in figure 2. It seems that salinity has a dual effect: Presence of salt in the reaction medium strongly inhibits the incorporation in roots grown in the absence of salts (line 3); In roots previously grown in saline media absence of salts in the reaction mixture allows a higher incorporation than in the presence of salts (line 2). The incorporation, however, was lower the higher was the salinity in the medium in which the roots were grown. It may be that roots grown in relatively low chloride concentrations show a slight adaptation, while those grown in sulphate salinized media show none (line 1). Roots grown in the presence of 1 atm NaCl show, in reaction mixture devoid of NaCl, incorporation ability even higher than the controls (line 2 in A).

The same experiments were repeated using leucine of higher specific activity (275 mc/mmole) and using a mixture of labeled amino acids (specific activity of 40 mc/m atom carbon). The results were virtually the same as those presented in figure 2.

It seemed of interest to follow the fate of the absorbed leucine in the tissue grown under various types of salinity. Only control plants and plants grown under 5 atm salts were studied. Total radioactive carbon absorbed was measured, as well as $^{14}CO_2$, ^{14}C in protein and ^{14}C in other fractions. The results are summarized in table I.

As can be seen from the results, salinity not only reduced the capacity of the roots to absorb external leucine, it also severely reduced the ability of the tissue to incorporate the amino acid into protein. Both types of salinity show a similar magnitude of the inhibitory effect. The fate of the remaining amino acid, that was absorbed but not incorporated into protein, differs in roots grown under different types of salinity. In roots grown under sulphate salinity more than 20 % of the total absorbed leucine was released as CO_2 while in roots grown under chloride salinity only 6 %.

As salinity reduced the ability of the tissue to incorporate leucine into protein, it seemed interesting to find out whether there is any difference in the amino acid composition of the proteins from roots grown under different conditions. Only one, preliminary, amino acid analysis has been made. This analysis has indicated that the nature of proteins synthesized in roots grown under different conditions may indeed be different.

Pea root tips are extremely rich in leucine, but leucine content is lower in salt-treated roots than in the controls. Also, roots grown under chloride salinity are richer in leucine than roots grown under sulfate salinity. Roots grown under sulfate salinity show a higher ammonia content than the 2 other treatments. Apparently they contain more labile, easily deaminated compounds, than the roots under the other treatments. Half cystine is highest in control roots and almost completely absent in sulfate treated roots. An unidentifiable substance, eluting on the fifty-first minute, appears only in roots treated with NaCl.

The effect of kinetin on incorporation of l-leucine into protein by normal and salt grown roots is shown in table II. The plants were grown in

Table I. *A Balance Sheet for ^{14}C Absorbed as ^{14}C-l-leucine-(U) in Pea Root Tips Grown in Presence and Absence of Salt*

The roots were grown in medium salinized either with NaCl or with Na_2SO_4 to 5 atm. The controls were grown in non-salinized Hoagland. Incorporation from medium of the same composition as growth medium.

Serial no.	Details	Non-salinized Hoagland	Hoagland salinized with NaCl	Hoagland salinized with Na_2SO_4
1	dpm in intact tips as measured after combustion	$5.15\pm0.23\times10^5$	$6.33\pm0.40\times10^4$	$1.34\pm0.41\times10^4$
2	dpm as released in CO_2	$2.83\pm0.68\times10^4$	$4.35\pm0.54\times10^3$	$2.99\pm0.1\times10^3$
3	Total absorbed (dpm 1+2)	$5.43\pm0.29\times10^5$	$6.76\pm0.40\times10^4$	$1.64\pm0.46\times10^4$
4	dpm in protein	$4.16\pm0.66\times10^5$	$3.92\pm0.53\times10^4$	$8.29\pm0.98\times10^3$
5	4 as % of 3	76.3 ±4.9	57.5 ±5.3	52 ±8.6
6	2 as % of 3	5.3 ±1.1	6.5 ±0.4	21.6 ±7.0
7	% of radioactivity in non-identified fractions: 100 − (5+6)	18	36	26

Table II. *Effect of Kinetin on Incorporation of l-leucine ^{14}C into Protein*

The plants were grown in medium salinized either with NaCl or with Na_2SO_4 to 5 atm. The controls were grown in non-salinized Hoagland. Incorporation into root tips was at the same salt concentration as in growth medium. Results are expressed by specific activity of treatment as percent of control.

Growth medium	Kinetin	Incorporation
	10^{-9} moles	%
Non-salinized Hoagland	— Kinetin	100
	+ Kinetin	65.5±4.0
Hoagland salinized	— Kinetin	19.1±2.5
with 5 atm NaCl	+ Kinetin	38.4±1.1
Hoagland salinized	— Kinetin	3.3±0.2
with 5 atm Na_2SO_4	+ Kinetin	0.9±0.1

Hoagland's nutrient media salinized to 5 atm with either NaCl or Na_2SO_4. l-leucine of the higher specific activity (275 mc/mmole) was used and 10^{-9} moles of kinetin were added to the reaction mixture. As can be seen from table II, kinetin significantly depressed the incorporation of leucine into protein, by the control roots and by roots exposed to 5 atm Na_2SO_4. However, kinetin stimulates significantly the incorporation in roots exposed to 5 atm NaCl. In 2 experiments the effect of kinetin on uptake was studied. In the presence of kinetin the uptake of l-leucine by control roots was only 80% of that in the absence of kinetin. In roots exposed to sulfate kinetin did not affect uptake at all, while in roots exposed to chloride, kinetin increased uptake by approximately 50%. It is evident therefore, that in those cases where there is a response to kinetin, incorporation is affected more than uptake.

Discussion

It is difficult to discuss the effect of salinization in general. Even our limited experiments using NaCl or Na_2SO_4 salinization show a number of effects on amino acid metabolism.

The presence of sodium chloride or sulfate in the incubation mixture considerably reduced amino acid uptake by the tissue; the depression by sulfate being more pronounced (table I). This is not due to a failure of the labeled amino acid to penetrate into the intercellular spaces as infiltrating the tissue with the reaction mixture did not affect the uptake.

Failure to take up the amino acid may therefore be due to changes in the permeability properties of the cells due to the presence of salinity.

It has been shown by Oaks (8, 9) that maize root tips do not synthesize all the amino acids required for protein synthesis and therefore depend on a supply of these from the upper part of the plant. If this is also the case for pea plants and since such transported amino acids also must pass cell walls and membranes, our system simulates the situation in entire roots.

In addition, to the effect on uptake, salinity also reduced incorporation of amino acids into protein. Here again sodium sulfate is more deleterious than sodium chloride, at iso-osmotic concentrations (table I, fig 1, fig 2).

In the presence of low salt concentration protein synthesis, under the experimental conditions used, approached saturation when about 0.5 μc/ml, were supplied. At the higher salt concentrations, however, incorporation was linear with increasing activity of the amino acid (fig 1). This may be taken to support the view that salinity affects permeability, thus affecting the concentration of the amino acid at the site of protein synthesis. Moreover the metabolic fate of the amino acid is also affected by the type of salinity and the concentration (table I).

The incorporation of the amino acid into protein, in absence of salt in the reaction mixture, is lower in roots grown at higher levels of salinity than in the control roots (table I and fig 1 and 2). This may be taken as an indication of salinity damage to the incorporation sites. However, roots grown at low levels of salinity showed even higher incorporation than the controls. Unfortunately, the absorptive ability of such roots was not studied. There is also some evidence for adaptation to NaCl salinity (fig 2).

The comparison of the 3 lines in the graphs in figure 2 indicates the possibility that, in root tissue, salinity affects uptake more than incorporation. This is supported also by the data presented in table I. These findings are contrary to those of Benzioni *et al.* (1) for tobacco leaf tissue. In their experiments, salt stressed leaf discs absorbed approximately three-fourths of the amount of labeled leucine absorbed by the non-stressed discs. In our experiments (table I), stressed roots absorbed only approximately one-tenth of the amount absorbed by the non-stressed roots. On the other hand the incorporation in roots was less affected by salinity than in leaves.

The balance of the absorbed amino acid (table I) shows clearly that various aspects of metabolism are affected by salinity and that the effects of NaCl and Na_2SO_4 are not identical. This is supported by the preliminary investigation of the amino acid composition of the proteins.

Working with tobacco leaves, Itai (4) and Benzioni *et al.* (1) have shown that kinetin can increase

incorporation of leucine into leaf proteins in non-stressed leaves and can abolish the inhibition in protein synthesis caused by various kinds of stress (water stress, osmotic stress, and salinity stress). It was suggested that reduction in kinetin content is the common denominator for most types of stress. It seems that this is not the case in our experiments (table II). While results with roots exposed to NaCl induced stress partially agree with those of Itai and Benzioni *et al.*, roots exposed to Na_2SO_4 induced stress behave differently.

Root tissue apparently differs from leaf tissue also in its response, or sensitivity, to kinetin treatment. While in Itai's (4) and Benzioni *et al.* (1) experiments, non-stressed leaf discs treated by kinetin, incorporated labeled leucine much more intensively than discs not treated with kinetin, this was not the case in roots. The same concentration of kinetin significantly inhibited uptake and incorporation in non-stressed root tissue.

In these experiments the effect of sodium salts of 2 different anions was studied. But the concentration of sodium itself at the 2 different treatments was not identical. Therefore the effect must be even more complicated than it appears at first sight. Much further work is necessary for a more complete clarification of the problem.

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