Phytochemistry, 1980, Vol. 19, pp. 1939–1942. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0901-1939 $02.00/0

EFFECT OF NaCl ON THE ACTIVITIES OF GLUTAMATE SYNTHASE FROM A HALOPHYTE *SUAEDA MARITIMA* AND FROM A GLYCOPHYTE *PHASEOLUS VULGARIS*

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(*Received* 4 *January* 1980)

Key Word Index—*Suaeda maritima*; Chenopodiaceae; *Phaseolus vulgaris*; Leguminosae; nitrogen metabolism; glutamate synthase; effect of NaCl.

Abstract—The action of NaCl on the activity of root and leaf glutamate synthase is compared in a halophyte, *Suaeda maritima* var. *macrocarpa* and in a glycophyte *Phaseolus vulgaris*. The addition of salt in the nutrient medium lowers the activity of glutamate synthase from *Phaseolus* without affecting that of *Suaeda*. This result, attributed to the fact that glutamate synthase is stimulated while glutamate dehydrogenase is partly inhibited in the halophyte grown in presence of high NaCl concentrations, suggests that the GS–GOGAT pathway is the primary route for ammonia assimilation. This pathway is especially active in the leaves. *In vitro*, NaCl (25–300 mM) reduces the activity of glutamate synthase in *Phaseolus* as well as in *Suaeda*. Comparison with results obtained *in situ* suggests that there are differences in intracellular compartmentalization between the two types of plant.

INTRODUCTION

Previous studies [1] have shown that the addition of sodium chloride in the nutrient medium is essential for the complete development of *Suaeda maritima* var. *macrocarpa*. Optimal growth requires a salt concentration in the order of 129 mM. This behavioural pattern raises the question of the operating possibilities of the assimilation routes of mineral nitrogen under extreme saline conditions. Comparison of results obtained in a glycophyte (*Phaseolus vulgaris*) and in the halophyte *Suaeda* [2] has shown that favourable conditions (0 mM NaCl for the bean and 129 mM for *Suaeda*) ensure maximal activity of glutamine synthetase (GS) and minimal activity of glutamate dehydrogenase (GDH). The glutamine synthetase–glutamate synthase route (GS–GOGAT) could therefore play a major role in ammonia assimilation in contrast to GDH, as it has been established for some higher plants [3]. In the halophyte NaCl would be required in order to achieve this predominance.

The aims of the present study were to determine whether the effects of salt on the levels of activities of root and leaf GOGAT from *Phaseolus* and *Suaeda* are compatible with this hypothesis.

RESULTS

Activity of glutamate synthase in leaves

Table 1 shows the principal characteristics of the glutamate synthase reaction in preparations from *Suaeda* leaves partially purified after desalting on a Sephadex G 25 column. Glutamate formation was found to be dependent upon the presence of both glutamine and α-ketoglutarate. Glutamine present alone in the reaction mixture will initiate some low glutaminase activity which must be subtracted from the total GS activity. Amino-oxyacetate inhibits contaminating transamination reactions; in the absence of the inhibitor, such reactions are responsible for the formation of some glutamate, resulting in an overestimation of the GOGAT activity. The inefficiency of pyridine nucleotides and the high glutamate synthesis observed in the presence of methyl viologen show that the measured activity is consistent with the reaction described by Lea and Miflin [4] in pea chloroplasts. It involves the activity of glutamate synthase (EC 1.4.1.7) and ferredoxin was shown by Lea and Miflin to be the specific electron donor *in situ*.

Table 1. Characteristics of the glutamate synthase reaction in leaves of *Suaeda maritima* grown in the presence of 129 mM NaCl

Reaction mixture	nmol glutamate/min
Complete with methyl viologen	38.7
minus amino-oxyacetate	39.4
minus α-ketoglutarate	2.3
minus glutamine	0.5
Complete with NAD(P)H	2.8

Activity of glutamate synthase in roots

Root extracts of *Suaeda* obtained after Sephadex G 25 treatment have a GOGAT activity dependent on the presence of both substrates (Table 2). In contrast to the previous case, pyridine nucleotides are more efficient electron donors than methyl viologen since they insure a two-fold increase of glutamate formation as opposed to that measured with methyl viologen. The utilization of NAD(P)H is however partly unrelated to the GOGAT activity since 25% of the total oxidation of both coenzymes

Table 2. Characteristics of the glutamate synthase reaction in roots of *Suaeda maritima* grown in the presence of 129 mM NaCl

Reaction mixture	Pyridine nucleotide oxidation (nmol/min)	Glutamate formation (nmol/min)
Complete with NADH (a)	8.6	12.1
minus α-ketoglutarate	2.6	0.6
minus glutamine	2.2	0.6
minus α-ketoglutarate–glutamine (b)	2.6	0
minus amino-oxyacetate	9.1	12.2
Complete with NADPH	8.0	11.5
Complete with methyl viologen	...	6
Glutamate synthase activity*	6	12

* Estimated by subtracting (b) from (a).

occurs in the absence of glutamine and ketoglutarate in the reaction mixture. This result demonstrates the presence of either a NAD(P)H oxidase or a NAD(P)H-dependent dehydrogenase in the root extracts. The specific glutamate synthase activity expressed as the rate of NAD(P)H oxidation was therefore calculated taking into account the interference due to these enzymes. In these conditions the stoichiometry of the reaction shows that two molecules of glutamate are formed for every molecule of NAD(P)H oxidized (6 nmol NADH oxidized for 12 nmol of glutamate formed per min). The measured activity appears to be very similar to the reaction catalyzed by the glutamate synthase (EC 2.6.1.53) described by various authors [5–7] in the roots of higher plants. The similar efficiency of NADH and NADPH as electron donors might be explained by a lack of specificity of the enzyme as Fowler *et al.* [6] and Dougall [8] have suggested or by the presence of a phosphatase converting NADPH into NADH [9].

Leaf and root extracts of *Suaeda* therefore show two types of glutamate synthase activities which were measured specifically after elimination of contaminating reactions. The same result is obtained with *Phaseolus*. In both cases formation of glutamate or oxidation of NAD(P)H were found to be proportional to the amount of enzyme extract and to the incubation time in the conditions defined in the Experimental.

Effect of NaCl *concentration in the nutrient medium*

Table 3 shows that glutamate synthase activity is much higher in the leaves than in the roots of the two species studied. This result is consistent with the observations of Marechal *et al.* [10] in *Phaseolus* and Stewart and Rhodes [11] in *Suaeda*. The addition of NaCl in the nutrient medium is followed by a 30% decrease of the GOGAT activity in the roots as well as in the leaves of the bean. In contrast, the level of activity of the enzyme remains constant in both types of tissues studied in *Suaeda*. This stability is maintained even for external salinities higher than those tested for *Phaseolus*. These results show that the ratio between root and leaf activities of a same plant is not modified by the presence of NaCl either for the halophyte or the glycophyte. The salt appears only to lower the potential of the GOGAT activity in the bean without affecting that of *Suaeda*. This last result based on experimental work on the action of NaCl completes the observations of Stewart and Rhodes [11], who indicated a stability of GOGAT activity in field grown *Suaeda* collected from soils with different salinities.

Effect of NaCl *concentration in the reaction mixture*

This study was carried out with leaf extracts of plants grown in presence of NaCl: 129 mM in the case of *Suaeda* and 51 mM in the case of *Phaseolus*. It was shown [12] that Sephadex G 25 treatment eliminates NaCl from GOGAT-rich fractions. Increasing salt increments were added in the reaction mixture resulting in saline concentrations ranging from 25 to 300 mM.

Figure 1 indicates that the GOGAT activity is reduced by half when NaCl concentration reaches 150 mM with the bean or *Suaeda*. It is already depressed by the addition of 25 and 50 mM of salt without significant differences in one species or the other. This same sensitivity to NaCl noted with the halophyte and the glycophyte has been previously reported for other enzymes [13–18] and was also indicated in the case of the GOGAT of the bacterial species *Aerobacter aerogenes* [19]. In contrast, Wallsgrove *et al.* [20] show a slight stimulation of the GOGAT activity from leaf extracts of *Vicia faba* when the incubation is realized in presence of 50 mM NaCl. This disagreement might be explained by different enzyme extraction and incubation conditions.

This *in vitro* study shows that the salt has a qualitative effect on GOGAT activity expressed similarly in the

Table 3. Effect of NaCl concentration in the nutrient medium on the glutamate synthase reaction in roots and leaves of *Phaseolus* and *Suaeda*

	Specific activities					
	Phaseolus			*Suaeda*		
NaCl (mM)	0	51	102	0	129	400
Roots	8.4	5.4	5.5	6.1	5.5	5.6
Leaves	96	82	68	69.3	69.2	68.5

Specific activity = nmol NADH oxidized/min/mg protein (in the case of roots) or nmol glutamate formed/min/mg protein (in the case of leaves).

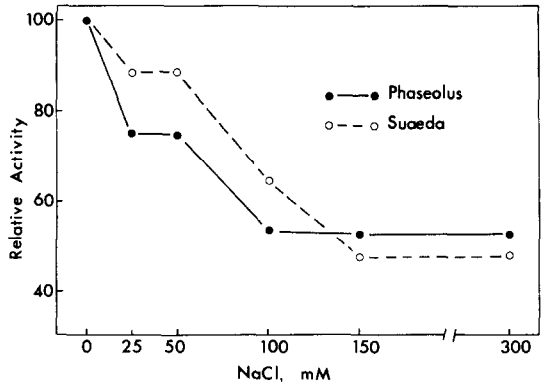

Fig. 1. Effect of NaCl concentration (mM) in the reaction mixture on glutamate synthase activity of the leaves of *Phaseolus* and *Suaeda*. Results expressed as percentages of activities measured without addition of salt in the reaction mixture.

halophyte and the glycophyte by an inhibition with all NaCl concentrations tested (25–300 mM). The conclusions are the same when extracts obtained from plants grown in the absence of salt are analysed.

CONCLUSION

It was previously established that the leaves of *Phaseolus* and *Suaeda* exhibited a high glutamine synthetase activity when both plants were grown in optimal saline conditions (129 mM NaCl for *Suaeda* and 0 mM for the bean). The results of the present study demonstrate that the enzyme potential of the GOGAT is sufficiently high in both cases to permit ammonia assimilation through the GS–GOGAT sequence. This sequence operates chiefly in leaf tissues where levels of glutamate synthase activity are much higher than in roots. This disparity in the distribution of GOGAT potential between roots and leaves is consistent with the results previously reported in some higher plants [10, 11].

The NaCl needed for the optimal growth of *Suaeda* favours this GS–GOGAT pathway instead of the glutamate dehydrogenase route, especially in the leaves. It was shown in fact [2] that optimal growth conditions (0 mM NaCl for the bean and 129 mM NaCl for *Suaeda*) induce maximal activity of glutamine synthetase and minimal activity of glutamate dehydrogenase in leaves. Contrasting salinities, therefore unfavourable conditions, lead to important glutamate dehydrogenase activity especially in roots [21]. The high stability of the leaf GOGAT demonstrated in *Suaeda* with respect to the NaCl present in the nutrient medium shows that the enzymatic potential of the enzyme can react to the stimulation of glutamine synthetase affected by NaCl. The salt would therefore be indispensable in the obligate halophyte for the optimal performance of the GS–GOGAT pathway, the ammonia assimilation route which is common to most higher plant glycophytes [3]. In contrast to *Suaeda* the addition of salt in the nutrient medium creates unfavourable conditions for the growth of *Phaseolus* and lowers the activity of this same enzymatic pathway.

The GOGAT activity decreases in the bean, as well as in *Suaeda*, when NaCl is present *in vitro*. The fact that this same sensitivity is not observed *in situ* in the halophyte suggests two hypotheses; even partially inhibited, the enzyme would be present in sufficient amount in the halophyte to permit good nitrogen assimilation at high salinities; or there would exist in *Suaeda* an enzyme protection system against the inhibition effect of NaCl which would be more efficient than in the glycophyte. This difference could be linked to the problem of intracellular compartmentalization and suggests the existence of modifications at the membrane level in the two types of plants.

EXPERIMENTAL

Plant material. Suaeda maritima var. *macrocarpa* and *Phaseolus vulgaris* were grown from seed. The growth conditions were as described previously [2]. Roots and leaves were collected after 21 days of growth in the case of *Phaseolus* and 45 days in the case of *Suaeda*. The intracellular mineral regulation phase characteristic of the latter species is then realized [22].

GOGAT extraction and purification. The analyses were carried out with *ca* 3 g freshly harvested tissue. The tissues were ground with a mortar and pestle at 2°. The extraction buffer consisted of 100 mM tricine–KOH, pH 7.5 with 5 mM EDTA, 12.5 mM mercaptoethanol, 1 mM phenylmethylsulfonylfluoride (PMSF). The extract was centrifuged at 37 000 *g* for 25 min. The supernatant was filtered and desalted on a Sephadex G 25 column equilibrated with a 0.1 M tricine–KOH buffer, pH 7.5, containing 12.5 mM mercaptoethanol. All the above operations were carried out at 4°. Protein in the enzyme preparation was estimated using the Folin phenol procedure of ref [23]. The cultures at different salinities (respectively 0, 51, 102 mM NaCl for *Phaseolus* and 0, 129, 400 mM for *Suaeda*) and each analysis was repeated at least 3 ×.

Enzyme assay. Enzymatic activities were assayed as described in ref. [5]. Glutamate synthase from leaves (EC 1.4.7.1) was determined by measuring glutamate formation. The reaction mixture contained in a final vol. of 0.7 ml; 5 mM glutamine; 5 mM ketoglutarate; 0.1 mg methyl viologen; 1.6 mg sodium dithionite; 1.6 mg $NaHCO_3$; 5 mM aminooxyacetate; 100 mM tricine–KOH buffer; the final pH was 7.5. After incubation at 30° for 20 min the glutamate formed was detected by PC and quantified by the method of ref. [24]. The activity of glutamate synthase from roots (EC 2.6.1.53) was expressed as the rate of NAD(P)H oxidation at 30°. The reaction mixture contained in a final vol. of 2.8 ml; 5 mM ketoglutarate; 5 mM aminooxyacetate; 0.16 mM NAD(P)H; 100 mM tricine–KOH buffer; the final pH was 7.5. In some assays, in addition to following NAD(P)H oxidation, the formation of glutamate resulting from glutamate synthase activity was detected by PC.

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