PROLINE AS A SOURCE OF NITROGEN IN PLANT METABOLISM

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SUMMARY

Branches of *Chenopodium album*, including leaves and young inflorescences, were put into a solution of ^{15}N-L-proline in order to absorb the latter with the transpiration stream. After 48 hrs of exposure a number of nitrogen fractions were separated. ^{15}N was found in free and bound amino acids, especially in arginine, alanine, glutamic and aspartic acid as well as in amino-lipids and chlorophyll fraction. Proline is considered to be an important source of nitrogen.

1. INTRODUCTION

A large amount of free proline was found in some local plant structures prior to their intensive growth, as, for instance, in growing points (BREYHAN *et al.* 1959; DURANTON & MAILLE 1962, DURZAN & STEWARD 1963), SHVEDSKAJA & KRUZHILIN 1966), and especially in pollen grains (TUPÝ 1964; BRITIKOV *et al.* 1964a; BRITIKOV & MUSATOVA 1964b; LINSKENS & TUPÝ 1966, LINSKENS & SCHRAUWEN 1969). Proline content in pollen grains of some species reaches about 2% of dry weight (BATHURST 1954), but sharply decreases after initiation of pollen germination (LINSKENS & SCHRAUWEN 1969). It was assumed (BRITIKOV *et al.* 1965, 1966) that proline is a potent reserve material which may be completely utilized in many ways, *e.g.*, a. for direct incorporation into structural and biologically active proteins, b. as a source of energy, c. as a source of nitrogen, and d. as a possible physico-chemical mediator of metabolism of the dormant and survival tissues. Some data obtained are in favour of the hypothesis that proline may be utilized for chlorophyll synthesis (DURANTON & MAILLE 1962; PERDRIZET & MACQUIRE 1963; BREYHAN & HEILINGER 1966).

The experiment presented here deals with proline as a possible source of nitrogen. According to the distribution of the labelled nitrogen atom from exogenously applied ^{15}N-L-proline, a direct confirmation of this idea was expected.

2. MATERIAL AND METHODS

^{15}N-L-proline (Schwarz Bioresearch, Inc., 98.9% purity, 95% ^{15}N excess) was applied to branches of *Chenopodium album* (young inflorescences with 3–4 leaves, 20 g total fresh weight) which were put into distilled water containing 50 mg of ^{15}N-L-proline (5.73 mg ^{15}N). Two parallel experiments were carried out. Microbiological contamination was prevented by addition of penicillin (20

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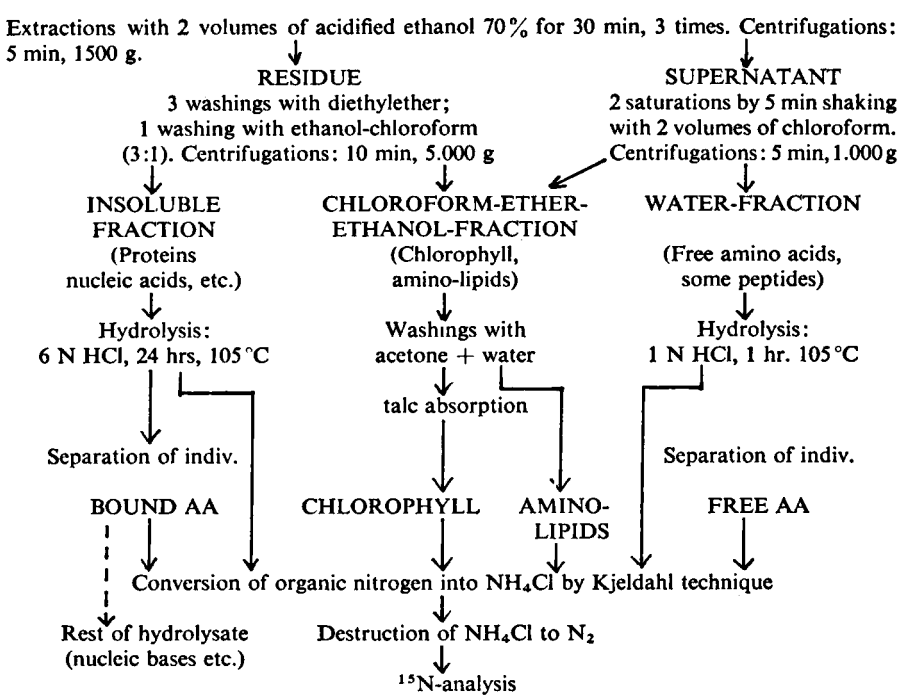
units/ml) and streptomycin (20 mg/ml). During the first 12 hrs of exposure (natural day length) the main part of the proline solution was absorbed; during the total 48 hrs of the main exposure 6 additional small portions of tap water were added and absorbed. After the exposure the basal parts of the branches were carefully washed to remove the traces of the proline solution, subsequently the whole tissue was fixed in liquid nitrogen, homogenized and fractionated according to *scheme 1*.

Scheme 1



Amino acids were separated using an automatic analyser as it has been recently described (LINSKENS & SCHRAUWEN 1969), and the individual amino acids were collected by a fraction collector.

Determinations of ^{15}N were made by a spectroscopic method (FAUST 1967). Eight discharge tubes (*fig. 1*) containing 5 mg CaO and CuO were preheated in the same time under vacuum (10^{-5} Torr) for 3 hrs at 500 °C. After cooling, turning the capillary into the discharge tube and sealing, the conversion from ammonium chloride into molecular nitrogen was completed within 16 hrs at 500 °C. Control estimations gave 100% conversion of NH_3 into $^{15}N_2$ in samples containing only 4 μg of total nitrogen. The difference between two parallel determinations was less than 1%. Spectrophotometric determinations were made with a ^{15}N analyser Isocommerz GMBH, Berlin, at the University of Groningen.

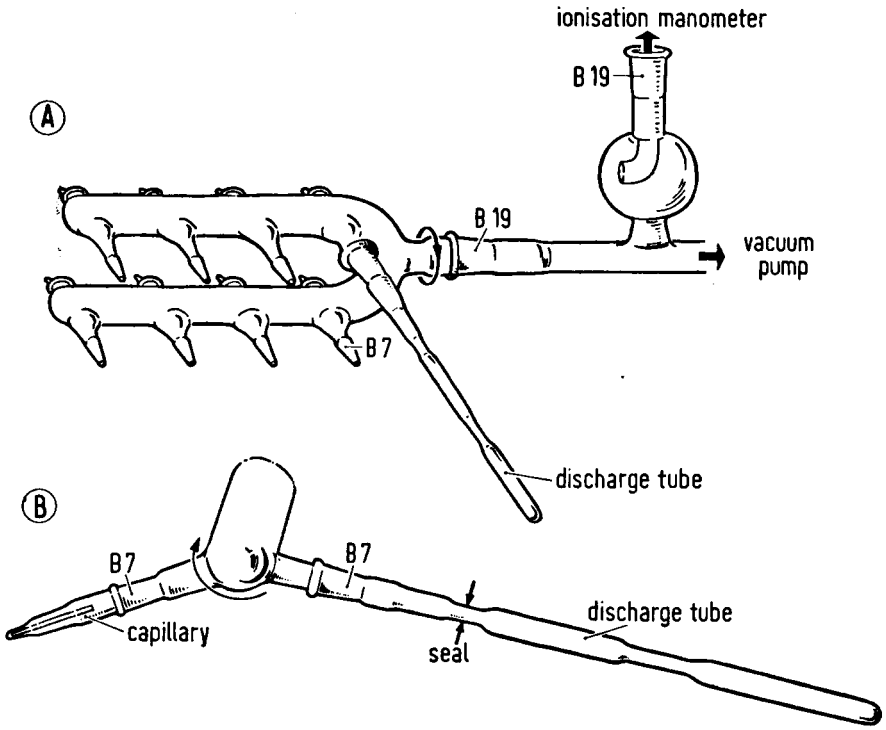


Fig. 1. A. Tube adaptor for simultaneous degassing of 8 discharge tubes, which contain CaO and CuO. When degassing was completed (3 hrs, 500 °C, 10^{-5} Torr) and the tubes were cooled down to room temperature, the adaptor was turned so that the capillaries simultaneously glided into their respective discharge tubes.
B. Side view (detail) of A. At ⚹ sealing of the tubes takes place, while the vacuum does not change.

3. RESULTS AND DISCUSSION

^{15}N-nitrogen from proline was found to be present in each fraction (*table 1*) and in all individual compounds analysed (*table 2*).

Non-isotopic automatic analysis only showed about 3 % of free proline in the tissues by the end of the exposure (48 hrs) from that absorbed by branches in a labelled form. It must also be mentioned that ^{15}N-excess of this rest part of free proline sharply decreased from about 95 to 23 atom per cent (*table 2*). This means that practically all exogenously absorbed ^{15}N-L-proline was degraded within the tissues and its nitrogen was released.

^{15}N-nitrogen from proline was found to be present in each investigated fraction (*table 1*) and in all individual compounds analysed (*table 2*).

The main portion of isotopic nitrogen was naturally found in free amino acids (FAA) as the first products of amination and transamination, and especially in those of them which are closely related to proline degradation and further transformations, *i.e.*, glutamic and aspartic acid, arginine, and maybe

Table 1. ^{15}N-excess in different fractions of *Chenopodium* tissues.

Fractions	^{15}N-atoms per cent
Free amino acids (FAA)	10.06*
Insoluble fraction (IF)	1.93*
Including:	
a. Bound amino acids (BAA)	1.03**
b. The other N-compounds (nucleic bases etc.)	0.90***
Amino-lipids	0.99
Chlorophyll	0.35

* estimated mass spetrometrically.
** Calculated from the data on ^{15}N-content and ^{15}N-excess for each BAA.
*** As the difference in ^{15}N-excess between IF- and BAA-fractions.

Table 2. Distribution of ^{15}N in the amino acids (in atom per cent).

Amino acids/fractions	free amino acids, FAA	bound amino acids, BAA
proline	23.0	3.78
glutamic acid	11.72*	1.27
aspartic acid		0.73
arginine		1.15
alanine		1.14
serine	2.35	1.40
glycine	0.73	0.30
Thr, Val, Met, Leu, Ileu Tyr, Phe, Lys, His (plus Orn, GABA, $EtNH_2$, for FAA, and Cys, Met for BAA)	2.72	0.49
NH_3 (amides, Try, free NH_3	7.40	0.98

* Calculated from the difference in ^{15}N-content between the whole FAA-fraction and the sum of all the rest FAA.

alanine as well as amides degraded to ammonia and the respective acids during hydrolysis (*table 2*). Unfortunately we failed to analyze these amino acids individually for ^{15}N. But since quantitative diminution of proline content was found to take place even after 48 hrs (*table 3*), and was accompanied by significant accumulation of arginine and aspartic acid, these latter really seemed to have a high enrichment with ^{15}N. Then, glutamic acid as a direct and transient intermediate of proline destruction might have the highest ^{15}N-enrichment without significant accumulation in the tissues.

Enrichment of the bound amino acids (BAA) was about 1/10 that of the free ones and had ^{15}N-excess as high as 1.03 atom per cent. This may be considered as significant, especially taking into account that protein turnover in the plant tissues is only 5–12% a day (HELLEBUST & BIDWELL 1964), and that endogenous non-isotopic nitrogen also took part in protein metabolism during the exposure.

Table 3. Changes in some free amino acids content with exposure (nitrogen-content in μg/10 g of fresh weight).

Amino acids	in 48 hrs	in 72 hrs	Per cent differences
proline	116	72	−37.1
arginine	197	245	+19.5
aspartic acid	82	98	+15.7
glutamic acid	179	185	+ 2.2
NH_3	232	249	+ 1.3
alanine	46	43	− 4.3
serine	34	35	+ 1.3

the rest amino acids: insufficient changes.

It is also of interest that bound amino acids only attract about a half of ^{15}N from the total insoluble fraction (*table 1*), the rest being lost in the columns of the AA-analyser. Nitrogen of this latter subfraction may represent first of all the nitrogen of nucleic acids, since aspartic acid, glycine, asparagine and glutamine are considered to be utilized in purine and pyrimidine synthesis (MEISTER 1965).

A fraction of the compounds soluble in acetone and insoluble in water (presumably amino-lipids) was also of high isotopic level, just as had been earlier observed in the experiments with ^{14}C-L-proline (BRITIKOV *et al.* 1966).

And finally, ^{15}N-nitrogen was also involved in chlorophyll synthesis.

Chenopodium album does not belong to the extravagant plants with a high natural content of proline or its derivatives in vegetative organs as e.g. *Citrus* or *Santalum* species do (GIRL *et al.* 1952). But this circumstance makes our conclusion on the important role of proline as a source of nitrogen for plant metabolism even more convincing.

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