Location of Several Enzymes of L-Arginine Catabolism in *Evernia prunastri* Thallus

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Agmatine ureo: hydrolase, which produces both putrescine and urea from agmatine, and urease, which hydrolyzes this urea, are highly restricted to the phycobiont cells in *E. prunastri* thallus. Arginase and L-arginine decarboxylase, which catabolize L-arginine, have a more extensive distribution between both symbiotic partners.

Introduction

The existence of polyamines as nitrogen storage substances has been investigated in several lichen species. Traces of ethanolamine have been found in *Lecanora myrinii* thallus [1]. Methylamines are common polyamines in *Lobaria* and *Sticta* species [2], being synthesized from glycine through a process which involves both oxidation and methylation reactions [3]. On another way, putrescine and agmatine have been found in *Platismatia glauca, Hypogymnia physodes* and *Pseudoevernia furfuracea* [4], although any investigations have been not carried out to explain their metabolic origin. In other plants, including bacteria, these polyamines are synthesized from L-arginine through a decarboxylation reaction to produce agmatine which is later hydrolyzed in both putrescine and urea [5]. Since urea can be considered as a key metabolite in the regulation of the symbiosis state in lichens [6–8], researchs on this metabolic pathway are very interesting. Control of symbiosis state involves the hydrolysis of urea by urease [7, 8], enzyme which has been found in *Cladonia vericillaris* [8], *Lobaria pulmonaria* [9] and *Parmelia roystonea* [10].

In the present paper studies on the location of L-arginine decarboxylase, agmatine ureo: hydrolase and urease have been performed in *E. prunastri* thallus as well as of an inducible arginase, recently found in the same lichen [11], which synthesizes urea by hydrolysis of L-arginine.

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Materials and Methods

E. prunastri collected from *Fagus sylvatica* in Montejo de la Sierra (Madrid) was used in this work.

Discs of thallus (1.0 g air-dried weight) were floated, at 26 °C in darkness, on 40 mM L-arginine in 0.1 M Tris-HCl buffer, pH 9.15, for 6 h for arginase assay or pH 7.2, for 4 h for L-arginine decarboxylase assay. Incubations for agmatine ureo: hydrolase analysis were carried out, for 8 h in darkness, on 40 mM L-arginine in 50 mM Tris-HCl buffer, pH 7.6, containing 1 mM EDTA and 1 mM dithiothreitol. For urease detection, the samples were floated for 7 h on 40 mM urea in 10 mM phosphate buffer, pH 6.9.

Isolation of both symbionts [12–14] was carried out by macerating the samples in a mortar with enough volume of the appropiate buffer containing 1 mM EDTA. Homogenate was centrifuged at $1000 \times g$ for 2 min and supernatant was again centrifuged for 1 min and 30 s until the hyphal suspension becomes colourless. Clear supernatant was then collected and tested at light microscope for the absence of algal cells. Green sediment was washed with the appropiate buffer and then centrifuged again. Drops of the pellet were tested at light microscope for the absence of fungal hyphae. Both supernatant and sediment were sonicated in a MSE disruptor for 1 min at 8000 microns with ice-cold protection, centrifuged at $17\,000 \times g$ for 15 min at 4 °C and used as cell-free extract for enzyme assays.

Protein was estimated by the Potty's method [15]. Urease was assayed by the Conway method [16] being 1.0 µmol of ammonia produced per mg of protein and minute a unit of specific activity. Arginase was assayed according to Greenberg method [17] modified by us [11], being a unit of specific activity 1 µmol of urea produced per mg of protein and minute. L-arginine decarboxylase was assayed by the conventional Warburg technique. The reaction mixture contained 0.78 mmol Tris-HCl, pH 7.2, 0.5 µmol $MgSO_4$, 0.1 µmol DTT, 4.0 nmol pyridoxal phosphate, 5.0 µmol L-arginine and protein to yield a final volume of 3.0 ml. Reaction mixtures were incubated at 26 °C for 20 min with gentle agitation. A unit of specific activity represents 1 µl of CO_2 produced per mg of protein and minute. Agmatine ureo: hydrolase was carried out as indicated by Morris and Pardee [18], measuring the urea formed by developped colour with diacetyl monoxime.

Table I. Distribution of enzymes of L-arginine catabolism in *Evernia prunastri* thallus. The ratio of total protein of mycobiont to phycobiont was, for all experiments, 15.66 ± 1.94. Values are the media of three replicates.

Enzyme	Thallus	Mycobiont		Phycobiont	
	(units of specific activity)	units of specific activity	% of intact thallus	units of specific activity	% of intact thallus
L-arginase	0.164 ± 0.02	0.054 ± 0.003	32.92	0.11 ± 0.01	67.08
L-arginine decarboxylase	0.72 ± 0.06	0.17 ± 0.02	23.61	0.55 ± 0.04	76.39
Agmatine ureo:hydrolase	0.16 ± 0.013	0.01 ± 0.001	6.25	0.15 ± 0.01	93.75
Urease	0.133 ± 0.02	0.01 ± 0.003	7.25	0.123 ± 0.01	92.48

Results and Discussion

Urease of *E. prunastri*, in terms of specific activity, is practically restricted to phycobiont cells whereas mycobiont shows only traces of this enzymatic activity. Similar results have been obtained for agmatine ureo:hydrolase, since 94 per cent of specific activity is restricted to the algal cells. The other two assayed enzymes, arginase and L-arginine decarboxylase, are more extensively distributed between both symbionts, although the phycobiont cells retain the most significant percentage of both enzymes, as specific activities, 67 per cent of arginase and 76 per cent of decarboxylase (Table I).

On the basis of these results, the phycobiont cells mainly conduct the synthesis of polyamines in *E. prunastri* but a substantial participation of the mycobiont in the catabolism of L-arginine cannot be excluded since the ratio of total protein of mycobiont/total protein of phycobiont was equal to 15.66. The following conclusions can be drawn. The enzymatic breakdown of L-arginine, which conduces to urea, gives, by hydrolysis of this compound, carbon dioxide which shall be fixed by photosynthesis in the phycobiont cells. In *E. prunastri*, 12 per cent of $^{14}CO_2$ produced from [^{14}C]urea is fixed and recovered as sugars, organic acids, amino acids and, specially, lichen phenols (unpublished results). The conversion of [^{14}C]urea to $^{14}CO_2$ followed by photosynthetic fixation has been also reported for free-living algae as *Chlorella* [19]. The low yield of photosynthesis, reported as characteristic for lichens [20], justifies that the enzymes of urea production as well as of urea hydrolysis, were preferably restricted to only one of the symbiotic partners, just that which supports the photosynthetic activity.

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