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REPRESSION OF ARGINASE AND AGMATINE AMIDINOHYDROLASE BY UREA IN THE LICHEN *EVERNIA PRUNASTRI*

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Key Word Index—*Evernia prunastri*; lichen; urea repression; arginase; agmatine amidinohydrolase; cyclic AMP.

Abstract—Two forms of arginase (EC 3.5.3.1) have been found in *Evernia prunastri*: (1) a light-arginase (M_r 180 000) induced by L-arginine—urea causes repression which is reversed by cyclic AMP; (2) a constitutive heavy-arginase (M_r 330 000) which is not affected by cyclic AMP. Agmatine amidinohydrolase (EC 3.5.3.11) is also repressed by urea but this effect is carried out at catabolite concentrations higher than those required to prevent the synthesis of the light-arginase. This repression is also relieved by cyclic AMP.

INTRODUCTION

Two main routes of urea biosynthesis have been found in the lichen *Evernia prunastri*. L-Arginine can be decarboxylated to produce agmatine [1], which is later hydrolysed to give putrescine and urea by an agmatine amidinohydrolase [2]. However, agmatine can also be hydrolysed by an agmatine iminohydrolase without production of urea [3]. Agmatine amidinohydrolase is produced by algal cells [4] whereas iminohydrolase is practically restricted to the mycobiont [3].

Alternatively, L-arginine can be hydrolysed by arginase to produce urea and L-ornithine [5]. Two forms of arginase have been found in *Evernia prunastri* thallus [6]. One of these, induced by L-arginine, has a M_r of 180 000 and an optimum pH at 9.0. The second form of the enzyme is not induced but activated by endogenous L-arginine and it has a M_r of *ca* 330 000 and an optimum pH at 6.5. These forms are named light- and heavy-arginase, respectively, on the basis of their M_rs. A constitutive arginase has also been detected in *Neurospora crassa* [7, 8].

In addition to light-arginase induction, L-arginine also induces both L-arginine decarboxylase and agmatine amidinohydrolase whereas urea behaves as a catabolite repressor of this hydrolase as well as of light-arginase [9]. It is well known that the addition of urea to bacterial cultures inhibits the expression of the operons sensitive to catabolite repression [10] which are also sensitive to nalidixic acid [11] and acridine orange [12] as transcriptional inhibitors. This is in contrast to the repression effected by glucose, which is relieved by cyclic AMP, even if the micro-organism does not produce this nucleotide [13]. Inhibition of these operons by urea in prokaryotes cannot be reversed by cyclic AMP.

In the present paper we attempt to determine the nature of the repression of both hydrolases, light-arginase and agmatine amidinohydrolase, by urea in *E. prunastri* and its dependence on cyclic AMP.

RESULTS AND DISCUSSION

The gene for the synthesis of light-arginase is subjected to catabolite repression, as shown in Table 1, since the addition of 100 mM glucose to the incubation medium containing 40 mM L-arginine in 0.1 M Tris–HCl buffer, pH 9.15, produces an arginase activity three times lower than that reached in the absence of the sugar. Glucose does not produce any effect on the activity of the purified enzyme. Addition of 0.5 mM cyclic AMP to the incubation medium completely reverses, and even stimulates, the synthesis of the enzyme. Measurements of enzyme activity were carried out after 6 hr of incubation.

Repression of light-arginase by urea is complete after 6 hr incubation on 40 mM L-arginine mixed with catabolite concentrations varying from 20 to 40 mM. However, by contrast with that found in prokaryotes [10], this repression is relieved by 0.5 mM cyclic AMP in the incubation medium. This reversal is complete when thallus samples are floated on 20 mM urea, partially achieved when 30 mM urea is used, and it is not reversed when 40 mM urea is added to arginine-containing media (Table 1).

As could be expected, neither 100 μM nalidixic acid nor 100 μM acridine orange affect the synthesis of light-arginase (data not shown). However, when thallus samples are maintained on buffer alone for 8 hr, arginase activity is developed, but *ca* 50% of this activity is light-arginase, the synthesis of which is enhanced by 0.5 mM cyclic AMP (Table 1). The other 50% of activity corresponds to heavy-arginase, which is not affected by 100 μM cycloheximide and which is not enhanced by cyclic AMP. A similar situation has been described for yeast invertase. A secreted, glycosylated enzyme is repressed by glucose [14] being synthesized by translation of a 1.8 kb RNA, the level of which is regulated by the sugar [15]. However, a second form of the enzyme, revealed as an intracellular, non-glycosylated protein, is translated from a 1.9 kb RNA, the level of which is not regulated by glucose.

Although agmatine amidinohydrolase behaves in a similar way, it also shows differences. The maximal activity is reached by thallus samples which have been floated for 8 hr on 40 mM L-arginine. Addition of 50 or 100 mM glucose to the incubation medium completely inhibits the appearance of enzyme activity. When these treatments are performed in the presence of 0.5 mM cyclic

Table 1. Effect of cyclic AMP on the differential rate of arginases and agmatine amidinohydrolase synthesis by *Evernia prunastri* thallus in the presence and absence of glucose or urea

Incubation medium	Arginase (units*)	Agmatine amidinohydrolase (milliunits*)
40 mM L-arginine†	16.5±1.1	1.72±0.1
40 mM L-arg+50 mM glucose		u.a.‡
40 mM L-arg+50 mM glucose+0.5 mM cAMP		1.57±0.1
40 mM L-arg+100 mM glucose	4.7±0.5	u.a.
40 mM L-arg+100 mM glucose+0.5 mM cAMP	21.6±1.9	u.a.
40 mM L-arg+20 mM urea	u.a.	1.83±0.1
40 mM L-arg+20 mM urea+0.5 mM cAMP	21.7±2.2	2.00±0.2
40 mM L-arg+30 mM urea	u.a.	
40 mM L-arg+30 mM urea+0.5 mM cAMP	9.8±0.7	
40 mM L-arg+40 mM urea	u.a.	1.50±0.1
40 mM L-arg+40 mM urea+0.5 mM cAMP	u.a.	1.75±0.2
40 mM L-arg+80 mM urea		u.a.
40 mM L-arg+80 mM urea+0.5 mM cAMP		1.20±0.1
0.1 M Tris–HCl, pH 9.15	13.4±0.8	
Buffer+0.5 cAMP	16.5±1.2	
Buffer+100 μM cycloheximide	7.2±0.6	
Buffer+100 μM cycloheximide+0.5 mM cAMP	8.1±0.9	

*A unit of enzyme activity is one μmol of urea produced per mg of protein per min.
†In all the cases, the data are the mean of three replicates, ± standard error.
‡u.a. is undetectable activity.

AMP, the values of hydrolase activity are almost identical to those obtained in the control assay when the repression is carried out by 50 mM glucose, but the nucleotide does not reverse the repression effected by 100 mM glucose (Table 1).

Urea behaves as catabolite repressor for concentrations higher than 40 mM. The activity is nullified by 80 mM urea and 0.5 mM cyclic AMP is able to reverse this effect, although partly when the repression is caused by the highest concentration of urea used here.

Reversal by cyclic AMP of the repression by glucose of light-arginase and agmatine amidinohydrolase seems to be in agreement with the scheme proposed by Peterkofsky [16] which implies the inactivation, derived from glucose transport, of an adenylcyclase. However, this does not explain the identical reversal when urea acts as a repressor of both enzymes, opposite to that found in prokaryotes [10] where, apparently, urea interacts with a specific sequence in the promoter. We can expect that the differences between the promoter in prokaryotes and promoter sequences in eukaryotes [17] were sufficient to explain this reversal. In addition, it is possible that the action of urea was carried out through an efflux of cyclic AMP outside the cells, although this hypothesis has been criticized by Buettner *et al.* [18]. In the same way, cyclic AMP can be displaced towards cell membranes and immobilized there thus preventing interaction with CAP [19].

On the other hand, heavy-arginase activity is not detected when urea is added to the media (Table 1) since this metabolite has been shown to be an uncompetitive inhibitor of this enzyme (unpublished results).

EXPERIMENTAL

E. prunastri (L.) Ach, growing on *Quercus rotundifolia* Lam, was collected in Valsaín (Segovia, Spain) and stored in polythene bags at 4° until required. Samples of 1 g of air-dried thallus were floated on 25 ml 40 mM L-arginine in 0.1 M Tris–HCl buffer, pH 9.15, for arginase induction, or on 25 ml of buffer alone. Incubations for agmatine amidinohydrolase analysis were carried out on 25 ml 40 mM L-arginine in 50 mM Tris–HCl buffer, pH 7.6, containing 1 mM EDTA and 1 mM dithiothreitol. Where indicated, 100 μM nalidixic acid, acridine orange or cycloheximide was added to the medium. All incubations were performed at 26° in the dark.

At different times, the samples were washed with H_2O and then macerated with a sufficient vol. of the same buffer used for each incubation to obtain a soln containing 1 mg of protein/ml. When heavy-arginase was assayed, 0.1 M Tris–HCl buffer, pH 6.5, was used to prepare the crude extract. The homogenates were centrifuged at 27000 *g* for 20 min at 4°. Supernatants were filtered through Millipore GS filters (0.22 μm pore diameter) and then dialysed against 4 l. of the appropriate buffer at 4°.

Protein was estimated by the method of ref. [20] using bovine serum albumin as a standard. Arginase was assayed according to the method of ref. [21] as modified in ref. [5]. A unit of sp. act. is 1 μmol of urea produced/mg protein per min, as well as the unit for agmatine amidinohydrolase activity, which was assayed as indicated in ref. [22].

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