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Activation of *Neurospora crassa* soluble adenylate cyclase by calmodulin

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The soluble form of adenylate cyclase was extracted and purified from wild-type *Neurospora crassa* mycelia. Brain or *N. crassa* calmodulin significantly enhanced this enzyme activity in assay mixtures containing Mg^{2+}–ATP as substrate. EGTA reverses this calmodulin activation.

Adenylate cyclase activity in *Neurospora crassa* and other lower eukaryotic organisms is dependent on Mn^{2+} (Flawiá & Torres, 1972; Paveto *et al.*, 1975; Varimo & Londesborough, 1976; de Silveira *et al.*, 1977; López-Gómez *et al.*, 1978). Evidence from this laboratory also indicates that in *N. crassa* mycelial strains the enzyme activity is only partially associated to sedimentable fractions. This adenylate cyclase can be obtained and purified from these strains as a soluble entity by procedures that do not require the use of detergents. Purified preparations of this enzyme activity are insensitive to fluoride and guanine nucleotides and highly dependent on Mn^{2+}–ATP; activity with Mg^{2+} is 1–2% of that found with Mn^{2+} (Reig *et al.*, 1982).

Hormone-sensitive adenylate cyclases in higher eukaryotic organisms are composed of at least three entities: the receptor, a protein which is called C (catalytic), and another protein which is called N (for nucleotide). In the absence of a functionally active N regulatory protein, as is the case in the AC^- S49 lymphoma variant, the catalytic component shows kinetic and molecular properties that are similar to those described for the *N. crassa* cyclase (Ross *et al.*, 1978). On the other hand, when this latter adenylate cyclase is partially associated to membranes it may interact with regulatory components extracted from avian erythrocyte membranes (Flawiá *et al.*, 1983). Calmodulin is also a known activator of some adenylate cyclase activities from different tissues (Van Eldik *et al.*, 1982), including the catalytic component in detergent-solubilized preparations from brain (Salter *et al.*, 1981). The presence of this modulator has been demonstrated in *N. crassa* (Ortega-Perez *et al.*, 1981; Cox *et al.*, 1982; Glikin *et al.*, 1982). Recent work by Rosenberg & Pall (1983) provided evidence of the existence in crude *Neurospora* mycelial extract of an adenylate cyclase activity that in the presence of Mg^{2+}–ATP is activated by guanine nucleotides; such an activity is not regulated by Ca^{2+}.

The present paper shows that purified preparations of *N. crassa* soluble cyclase activity may be stimulated by *N. crassa* and brain calmodulins in assays performed with Mg^{2+}–ATP as substrate.

Experimental

Cultures of St. Lawrence 74 wild-type *Neurospora crassa* strain were performed in Vogel's minimal medium as described elsewhere (Reig *et al.*, 1982). *N. crassa* soluble adenylate cyclase activity was purified up to the step of Bio-Gel A-5m column chromatography (Reig *et al.*, 1982). Calmodulin from *N. crassa* mycelia or brain cortex was purified by chromatographies on DEAE-cellulose, hydroxyapatite and Bio-Gel P-60 as previously described (Cox *et al.*, 1982; Glikin *et al.*, 1982), but with buffer solutions that did not contain EGTA. When analysed by electrophoresis on 12.5%-polyacrylamide gel slabs, in the presence of sodium dodecyl sulphate, both preparations were homogeneous. Adenylate cyclase activity was assayed in the presence of 2mM-$MgCl_2$ as described elsewhere (Flawiá *et al.*, 1983).

Results and discussion

Fig. 1 shows that calmodulin increased about 8-fold the specific activity of *Neurospora* adenylate cyclase in a soluble preparation. Since calmodulin was purified by a method that precludes the use of

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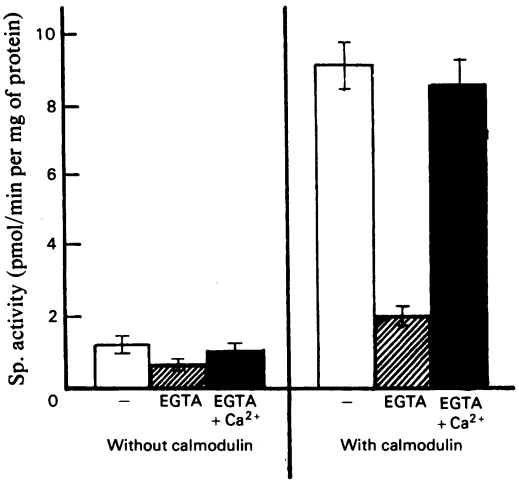

Fig. 1. *Effect of N. crassa calmodulin and EGTA on soluble adenylate cyclase activity in N. crassa*
The assay mixtures contained 10 μg of protein of soluble adenylate cyclase preparation and 30 ng of purified *N. crassa* calmodulin. Data show the means ± S.E.M. for three different experiments. EGTA and $CaCl_2$ concentrations were 50 μM and 150 μM respectively. Other conditions are described in the Experimental section.

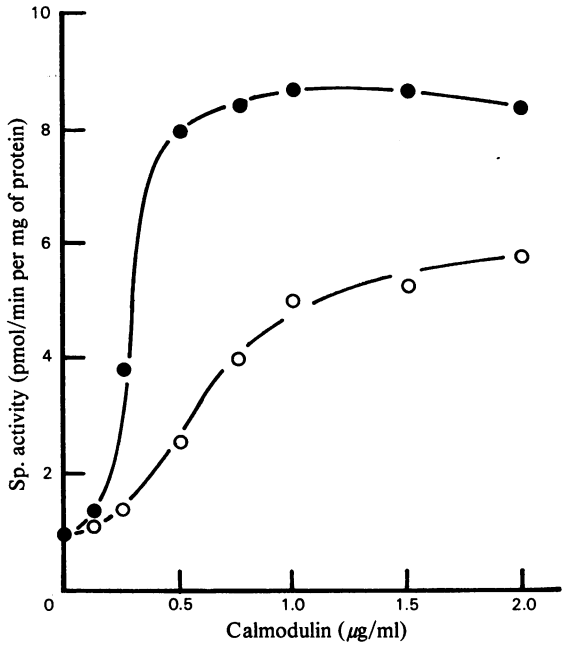

Fig. 2. *Activation of N. crassa soluble adenylate cyclase by N. crassa and brain calmodulins*
Reaction mixtures contained 2 mM-Mg^{2+} and the indicated amounts of *N. crassa* (●) or brain (○) calmodulin. Other conditions are described in the Experimental section.

EGTA in extraction and chromatography buffer solutions, it is possible that the factor preparations contained Ca^{2+}. This may explain the stimulation of adenylate cyclase activity by calmodulin in the absence of added Ca^{2+}. In any case, activation by calmodulin was blocked by EGTA, and the addition of Ca^{2+} to the mixtures containing the chelating agent restored the activation. The effects of EGTA and Ca^{2+} in the absence of calmodulin were negligible.

In addition, with the use of Ca^{2+}/EGTA buffers half-maximal stimulation of adenylate cyclase was observed at about 0.5 μM 'free' Ca^{2+}. Furthermore, adenylate cyclase of *N. crassa* is more active with Mn^{2+}–ATP as substrate (Reig *et al.*, 1982), but under these conditions the addition of calmodulin did not modify the activity (results not shown). Dose-dependence of *N. crassa* soluble adenylate cyclase activity on calmodulin is shown in Fig. 2. Both *Neurospora* and brain calmodulins activated the enzyme in assays supplemented with Mg^{2+}. The fungal factor, however, seemed more efficient in bringing about stimulation of cyclase activity, since the apparent affinity and the maximal activation reached by *N. crassa* calmodulin were greater than with the brain factor. Half-maximal stimulation with *N. crassa* calmodulin was obtained with about 300 ng/ml. Those results indicate that *Neurospora* adenylate cyclase might be under the control of intracellular Ca^{2+} concentrations through its interaction with calmodulin. In addition, evidence is given in this paper providing further support on the similarities between the catalytic component of adenylate cyclases from lower and higher eukaryotic organisms.

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