Polyamine, Magnesium and Ribonucleic Acid Levels in Steady-state Cultures of the Mould *Aspergillus nidulans*

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Results from experiments *in vitro* strongly suggest that major roles can be ascribed to polyamines in controlling the stability, activity and synthesis of ribonucleic acids. Furthermore, functional substitution of polyamines for inorganic cations, particularly magnesium ions, in cell-free protein synthesis is well substantiated (see Cohen, 1971). Recently we have been analysing the effects of culture conditions on the chemical composition of *Aspergillus nidulans* and have found fluctuations in polyamine and magnesium concentrations in response to a changing environment, while biomass and RNA remained constant. This paper describes the influence of steady-state growth rate on hyphal concentrations of spermidine, spermine and Mg^{2+} ions.

METHODS

A hyaline mutant, 13 mel, of *Aspergillus nidulans* (Bull & Faulkner, 1965) was grown in a chemically defined liquid medium (Carter & Bull, 1969), the Mg^{2+} concentration of which was 1 mM. Continuous-flow cultures were made in a 3.2 l fermenter unit (L.H. Engineering Ltd), the specifications and operation of which have been detailed by Rowley & Bull (1973). The fermenter was used in the chemostat mode, the carbon and energy source, glucose, being growth-limiting. The maximum specific growth rate of the mould in the defined medium was 0.20 h^{-1} and analyses were made at the following steady-state dilution rates: 0.02, 0.05, 0.07, 0.10, 0.125 and 0.175 h^{-1} . At one dilution rate (0.02 h^{-1}) the culture was heterogeneous and comprised vegetative hyphae, conidiophores and free conidia.

Mycelia, thoroughly washed with 0.11 M-phosphate buffered saline, pH 6.8, were freeze-dried and macerated at 0 °C in 0.2 N-perchloric acid (5 mg mycelium ml^{-1}) by a motor-driven Teflon-glass homogenizer (six passes at 750 rev./min for 10 s). Analysis of the saline washings revealed that neither polyamines nor magnesium ions had been leached from the mycelia. Polyamines in the perchloric acid extract were assayed by the method of Dion & Herbst (1970). The fluorescent dansyl derivatives were separated on silica-gel thin-layer chromatography plates (Eastman Kodak Co.), eluted by cutting out the spots and extracting the silica gel three times with an ethyl acetate-cyclohexane mixture (2:3, v/v) and their fluorescence measured in a Perkin Elmer MPF-3 fluorescence spectrometer (wavelength of activating light 365 nm, slit 8; fluorescence measured at 520 nm, slit 8). Magnesium was measured by the Titan-Yellow method (Herbert, Phipps & Strange, 1971) and three-times extracted macerated mycelium (0.2 N- HClO_4 at 70 °C) was assayed for RNA by the Schneider orcinol procedure (Herbert, Phipps & Strange, 1971). Spermidine, spermine (Sigma) and yeast RNA (BDH Ltd) were used as reference compounds.

Fig. 1. The influence of dilution rate on hyphal contents of spermidine, spermine, Mg²⁺ ions and total ribonucleic acid. (a) Spermidine (○), spermine (●), Mg²⁺ (△). (b) Total polyamines (solid histograms), Mg²⁺ (open histograms), total RNA (○). All values are of steady-state concentrations.

RESULTS AND DISCUSSION

Mycelial concentrations of magnesium, spermidine and spermine as a function of growth rate are shown in Fig. 1(a). Magnesium concentrations varied nearly sixfold (18 to 97.5 μmol/g dry weight) and fell dramatically with increasing dilution rate. An average value of 97.5 μmol/g dry weight for the Mg²⁺ concentration of vegetative bacteria is evident from Tempest's (1969) data and this is equivalent to an approximate molar concentration of 150 mM. We have not estimated the internal water of *Aspergillus nidulans* mycelia, but using the value obtained by Viotti, Bagni, Sturani & Alberghina (1971) for young mycelia of *Neurospora crassa*, the magnesium concentration of *A. nidulans* varied over the approximate range 6 to 37 mM. The Mg²⁺ concentration of exponentially growing *N. crassa* was 16 mM (Viotti *et al.* 1971). In contrast to Mg²⁺, spermidine and spermine concentrations of *A. nidulans* increased as the dilution rate was raised; spermidine increased from 6 μmol/g dry weight (2.9 mM) to 32 μmol/g dry weight (15.4 mM) and spermine from 1.0 μmol/g dry weight (0.40 mM) to 1.5 μmol/g dry weight (0.59 mM). Comparable values for exponentially

growing *N. crassa* were 16 mM and 0.22 mM, respectively (Viotti *et al.* 1971). These data take on a special significance when they are related to the steady-state RNA concentrations in the mycelia. The molar ratio of Mg^{2+} to RNA does not vary with dilution rate in carbon-limited cultures of bacteria (see Tempest, 1969) but our data from *Aspergillus nidulans* show marked variations (Fig. 1*b*). However, when the polyamines are considered, the molar ratio of polyamines plus Mg^{2+} to RNA remained constant at approx. 2:1. It seems therefore that the synthesis of the polyamines, and spermidine in particular, changes in response to growth-rate dependent fluctuations in Mg^{2+} in such a way that the cation to RNA ratio is maintained. Whether this ratio is critical for the stability of ribosome complexes or for RNA synthesis, and to what extent polyamines are functionally interchangeable with Mg^{2+} ions, are as yet unanswered questions. The ratio of total cation (polyamine plus Mg^{2+}) to RNA remained constant only under conditions of vegetative growth and changed significantly in the heterogeneous culture of spores and mycelium (dilution rate = $0.02\ h^{-1}$). This effect would seem predictable when one considers the large turnover of RNA during conidiation (Righelato, Trinci, Pirt & Peat, 1968) and the change in ribosome efficiency which occurred when the dilution rate was decreased to $0.02\ h^{-1}$ (Bushell, McGetrick & Bull, 1973).

Why the internal Mg^{2+} concentration falls with increasing dilution rate also requires investigation. Perhaps this reflects a progressively impaired magnesium-assimilation mechanism, as preliminary chemical analyses of the hyphal wall (A. M. T. McGetrick & A. T. Bull, unpublished data) have revealed that its net negative charge and uronic acid content also decreased as the dilution rate was raised. How much, if any, of the total mycelial Mg^{2+} and polyamines are bound to the wall polyuronates has not been determined but further analyses of this type are to be made.

The chemostat provides an ideal system for investigating interrelated phenotypic changes in microbial chemistry and function. Magnesium-limited chemostats are currently being used for further resolving the changes reported in this paper.

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