Pa I I, hl. L. Blasticidin-S: on inhibitor of protein synthesis in Neurospora.

Cycloheximide has been used widely as an inhibitor of protein synthesis in Neurospora. It is difficult to eliminate the possibility that results obtained with cycloheximide or other antibiotics may be due to secondary effect* independent of the inhibition of protein synthesis. This difficulty maker it desirable to have alternative antibiotics which effectively inhibit protein synthesis in Neurospora for comparison.

This note concerns a study of the antibiotic, blasticidin-S, on two-day-old mycelial pads of wild type strain ST74A, grown as described previously (Pall 1970 Biochim. Biophys. Acta 203:139). Pods were shaken with blasticidin-S for various periods of time before being shaken with 1 μC L-^{3}H lysine for 2 minutes. The mycelial pods were then washed, extracted with 5 % TCA and the uptake and incorporation into protein (hot TCA insoluble, NaOH soluble fraction) were measured. As shown in Table 1, even a half-minute preincubation with blasticidin-S giver significant inhibition of incorporation into protein. A ten minute preincubation with 50 μg/ml blasticidin-S monohydrochloride giver almost complete inhibition of incorporation. Thus blasticidin-S is rapidly effective in inhibiting lysine incorporation into protein. Cycloheximids inhibits incorporation even more rapidly than doer blarticidin-S, a half-minute preincubotion with 10 μg/ml cycloheximide inhibiting incorporation by 98 %.

Blasticidin-S, under the above condition*, shows little effect (<20 %) on lyrine uptake. The amino acid pool, as measured by cold TCA extractable ninhydrin positive material, shows little (0-30 %) increase in the presence of blasticidin-S. Consequently the inhibition of incorporation into protein would be expected to be a gwd measure of the inhibition of protein synthesis. Other experiment* showed that a IO-minute preincubation of pads with 50 μg/ml blasticidin-S monohydrochloride had little or no (00%) effect on the rate of uridine uptake or its incorporation into nucleic acid. The results support the conclusion that blasticidin-S is a rapid, relatively specific inhibitor of protein synthesis in Neurospora.

The blasticidin-S monohydrochloride was manufactured by the Kaken Chemical Co., Ltd., Japan and was a gift of Marubeni-Lida (America), Inc., San Francisco. The author would be happy to supply samples of blasticidin-S monohydrochloride to interested investigators.

Table 1. Inhibition of lysine incorporation into protein.

Time of preincubotion	Antibiotic used	% Inhibition of incorpomtion
1/2 min.	1 μg/ml blasticidin-S. HCl	18
1/2 min.	10 μg/ml blasticidin-S. HCl	72
1/2 min.	50 μg/ml blasticidin-S. HCl	86
1/2 min.	10 μg/ml cycloheximide	98.2
10 min.	1 μg/ml blasticidin-S. HCl	68
10 min.	10 μg/ml blasticidin-S. HCl	93.9
10 min.	50 μg/ml blasticidin-S. HCl	99.0

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