EVIDENCE AGAINST A GENERAL ROLE OF NADP-GLYCOHYDROLASE IN DIFFERENTIATION OF *STREPTOMYCES GRISEUS*

Sir:

Organism-specific bioregulators (autoregulators) have been shown to play a crucial role in triggering the onset of morphological changes and antibiotic production in streptomycetes[1~3], but, so far, the biochemical mechanism of their action is poorly understood. The inducing effect of 'A-factor' on the formation of aerial mycelium and production of streptomycin by *Actinomyces streptomycini* is postulated to involve NADP-glycohydrolase (E. C. 3. 2. 2. 6)[4]. A-factor was reported to stimulate the formation of NADP-glycohydrolase in Str$^-$Amy$^-$ mutants and the product, phospho-adenosinediphosphoribose, was found to act as a competitive inhibitor of the enzymes of carbohydrate catabolism[4] and the citrate cycle[5] during development of aerial mycelium. It was the aim of the present work to determine whether this mechanism of control by NADP-glycohydrolase exists in other streptomycetes. As a model we used *Streptomyces griseus* JA 5142 forming both aerial mycelium and leukaemomycin, a daunomycin-type anthracycline antibiotic[6]. This strain (Amy$^+$ Lkm$^+$) was compared with two antibiotic-blocked and non-aerial-mycelium-forming mutants, JA 5142/39 and JA 5142/86 (Amy$^-$ Lkm$^-$ strains)[7].

Strain JA 5142/86 has been shown to produce both aerial mycelium and antibiotic in surface cultures upon addition of a novel bioregulator[7]. The bioregulator, distinguished from A-factor by chromatographic properties and preliminary results of physicochemical analysis, was recently isolated from the culture medium of different strains of *Streptomyces griseus* (GRÄFE *et al.*, to be published). When it was added at zero time to agar cultures of strain JA 5142/86, and the cultures incubated at 28°C, development of aerial mycelium (Fig. 1) and production of leukaemomycin became evident by 48 hours. Cultures of strain JA 5142/86 with and without addition of purified bioregulator of strain JA 5142 and of strain JA 5142/39 were grown separately under the same conditions and on the same medium[7]. After appropriate intervals, the surface mycelia were scraped from the cellophane-coated agar and homogenized by 90-second and sonic disruption at 0°C in 0.05 M tris buffer, pH 7.2 (Labsonic 1510, Braun Melsungen, F.R.G.). After 15 minutes centrifugation (23,000$\times g$, 0°C), the supernatant was used for the spectrophotometric assay of NADP-glycohydrolase according to ZATMAN *et al.*[8] with the modifications reported[5]. The test mixture contained in a volume of 1.125 ml; 0.1 M tris buffer pH 7.4, 1 mM NADP and mycelium extract (approximately 0.02 mg of protein). Incubation time was 10 minutes (35°C). Formation of the cyanide complex of intact NADP was measured at 340 nm. Fig. 2 shows the specific activities of the enzyme in the mycelium. Both the original strain, *Streptomyces griseus* JA 5142 (Amy$^+$ Lkm$^+$), and its Amy$^-$ Lkm$^-$ mutant, JA 5142/39, possessed high levels

Fig. 1. Stimulatory effect of zero-time additions of the novel bioregulator on formation of aerial mycelium by agar-plate cultures of *Streptomyces griseus* JA 5142/86 after 4 days incubation at 28°C.
a) 0.5 mcg, b) 1 mcg and c) 5 mcg of bioregulator.

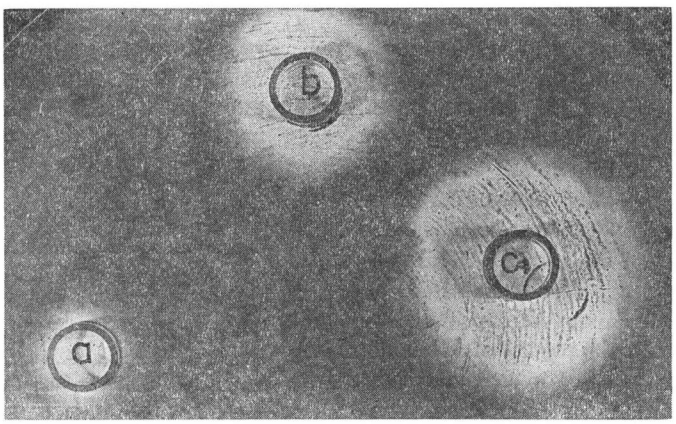
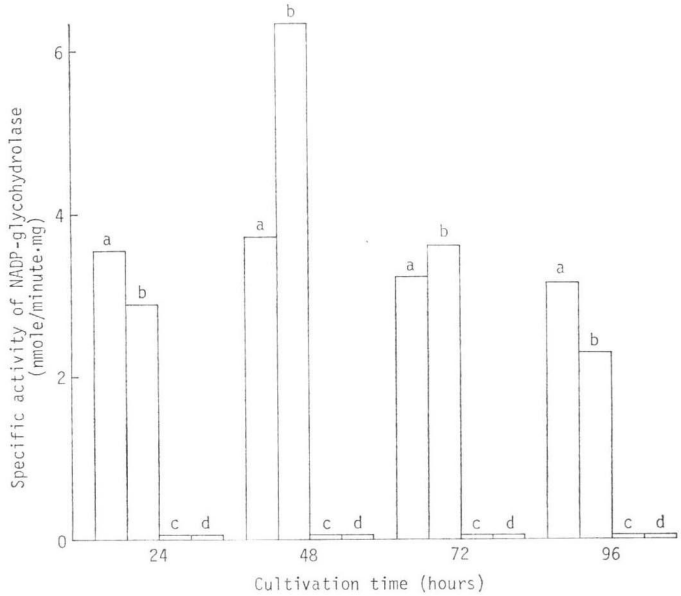


Fig. 2. Mycelial specific activity of NADP-glycohydrolase during agar-plate cultivation of *Streptomyces griseus*.

a) JA 5142 (original strain; Amy$^+$ Lkm$^+$), b) JA 5142/39 (Amy$^-$ Lkm$^-$ mutant), c) JA 5142/86 (Amy$^-$ Lkm$^-$ mutant), d) JA 5142/86+5 mcg of bioregulator per cm^2 of agar surface added at zero time. The activities of NADP-glycohydrolase are expressed as μmole/minute·mg of extracted mycelial protein.



of NADP-glycohydrolase while strain JA 5142/86 (Amy$^-$ Lkm$^-$) lacked appreciable activity. In contrast to the known effect of the A-factor on Amy$^-$ strains of *Actinomyces streptomycini*[1,4] there was no stimulation of enzyme production in strain JA 5142/86 by adding the novel bioregulator. With the supplement the mutant developed normal aerial mycelium and produced leukaemomycin as shown by bioassay and TLC.

Discussion

The findings presented above indicate that, in leukaemomycin-producing strains of *Streptomyces griseus*, there is no correlation between cellular differentiation and the production of NADP-glycohydrolase. In contrast to the situation in *Actinomyces streptomycini*, the formation of both aerial mycelium and antibiotic in *Streptomyces griseus* JA 5142/86 seems not to be coupled to induction of the enzyme. This suggests that metabolic regulation mediated by NADP-glycohydrolase cannot be considered as a general mechanism for the control of differentiation and antibiotic formation in streptomycetes that produce this enzyme.

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References

1) KHOKHLOV, A. S.; L.N. ANISOVA, I.I. TOVAROVA, E. M. KLEINER, I. V. KOVALENKO, O. I. KRASILNIKOVA, E. Y. KORNITSKAYA & S. A. PLINER: Effect of A-factor on the growth of asporogenous mutants of *Streptomyces griseus* not producing this factor. Z. Allg. Mikrobiol. 13: 647~655, 1973
2) POGELL, B. M.: Regulation of aerial mycelium formation in streptomycetes. Proc. Int. Symp. on Genetics of Industrial Microorganisms (SEBEK, O. K. & LASKIN, A. I.; *eds.*), Amer. Soc. Microbiol. 218~224, 1979
3) MARTIN, J. F. & A. L. DEMAIN: Control of antibiotic biosynthesis. Microbiol. Rev. 44: 230~251, 1980

4) VORONINA, O. I.; I. I. TOVAROVA & A. S. KHOKHLOV: Studies on the A-factor induced inhibition of glucose-6-phosphate dehydrogenase in *Actinomyces streptomycini*. Bioorg. Khim. (Moscow) 4: 1538~1546, 1978

5) GRÄFE, U.; E. J. BORMANN & G. TRUCKENBRODT: Control by phospho-adenosinediphosphoribose of NADP-dependent isocitrate dehydrogenase and 6-phosphogluconate dehydrogenase in *Streptomyces griseus*. Z. Allg. Mikrobiol. 20: 607~611, 1980

6) FLECK, W. F. & D. G. STRAUSS: Leukaemomycin, an antibiotic with antitumor activity. 1. Screening, fermentation and biological activity. Z. Allg. Mikrobiol. 15: 495~503, 1975

7) ERITT, I.; U. GRÄFE & W. F. FLECK: A screening method for bioregulators in anthracycline-producing streptomycetes. Z. Allg. Mikrobiol. (in press)