NOTE

Effect of S-Adenosylmethionine on Antibiotic Production in *Streptomyces griseus* and *Streptomyces griseoflavus*

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S-Adenosylmethionine caused overproduction of streptomycin (by *Streptomyces griseus*) and bicozamycin (by *Streptomyces griseoflavus*) when added to the medium at an appropriate concentration. The enhancement of streptomycin production was accompanied by a 1.35-fold increase in transcript of *strR* (a pathway-specific positive regulatory gene), and a 1.9-fold increase in activity of streptomycin kinase (an enzyme for streptomycin biosynthesis). These results, together with the previous works dealing with *Streptomyces coelicolor* (J. Bacteriol. 185, 601–609, 2003) and *Streptomyces lividans* (J. Bacteriol. 185, 592–600, 2003), implicate the significance of S-adenosylmethionine as an intracellular signal molecule for onset of secondary metabolism in the *Streptomyces* species.

S-Adenosylmethionine (SAM) serves as a methyl donor for the methylation of cytosine and adenosine bases in DNA, rRNA, and tRNA, various proteins, and small molecules which are important in both prokaryotic and eukaryotic organisms^{1–4}. Working with certain mutant strains (ND mutant) derived from *Streptomyces coelicolor* by selecting for low level resistance to streptomycin, we recently reported the significance for the level of intracellular SAM in initiating antibiotic production⁵. Namely, introduction of a high-copy-number plasmid containing the *metK* gene (which codes for SAM synthetase) into wild-type *S. coelicolor* cells resulted in the hyperproduction of actinorhodin. Furthermore, addition of SAM to a culture medium induced actinorhodin biosynthesis in wild-type *S. coelicolor*. Since overexpression of *metK* stimulated expression of the pathway-specific regulatory gene *actII-ORF4* (as demonstrated by the RNase protection assay), we concluded that SAM plays a role in the production of antibiotics by initiating secondary metabolic processes. In addition to the positive effect on antibiotic production, SAM also negatively affected morphological differentiation in *Streptomyces lividans* as demonstrated by Kim *et al.*⁶, which is supported by a previous report indicating that sporulation of *B. subtilis* is prevented by SAM⁷. Here, we demonstrate that SAM is effective in enhancing antibiotic production in other members of *Streptomyces*, *S. griseus* IFO13189 (which produces streptomycin) and *S. griseoflavus* FERM1805 (which produces bicozamycin).

Effect of SAM on antibiotic production and growth.

We previously reported that addition of SAM was effective in inducing hyperproduction of streptomycin in *S. griseus* when added (at 1 mM) at the time of inoculation⁵.

This was confirmed using a synthetic and nutrient medium SPY⁸, in which the production of streptomycin increased 3.6-fold and 3-fold, respectively (Fig. 1). However, the

Fig. 1. Effect of SAM on growth and streptomycin production in *S. griseus*.

The *S. griseus* wild-type strain IFO13189 was grown in a synthetic medium (A) or SPY medium (B) as described previously⁸. SAM (1 mM) was added at the time of inoculation (○) or at 24 hr (□) and 48 hr (△) [in A], or at 8 hr (□) and 13 hr (△) [in B], and incubation was further continued for the indicated times. The closed symbol (●) represents no addition of SAM. Streptomycin was determined by bioassay using *Bacillus subtilis* ATCC6633 as a test organism.

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efficacy of SAM was less pronounced when SAM was added at late growth phase (*i.e.* 24–48 hr in synthetic medium or 8–13 hr in SPY medium). The growth rate (and also final biomass) was not affected by the addition of SAM, regardless of the addition time (Fig. 1). To assess the possibility that effects observed were a result of metabolic product(s) generated from SAM rather than being directly attributable to the activity of SAM, we tested the effects of methionine and adenosine on streptomycin production in *S. griseus*. These metabolites (added at 1 mM) showed virtually no effect on streptomycin production (data not shown), suggesting that SAM is indeed directly responsible for the enhanced production of streptomycin.

Working with an arginine-auxotrophic mutant of *S. griseoflavus* FERM1805, Ochi *et al.* previously reported that exogenous addition of SAM partially corrects a defect in bicozamycin production⁹. Therefore, we studied the effect of SAM on antibiotic production in the wild-type strain FERM1805. Like streptomycin production in *S. griseus*, the production of bicozamycin increased 2-fold when SAM was added 24 hr after cell inoculation (Fig. 2) without affecting growth. Thus, exogenously added SAM enhanced the production of antibiotics in both *S. griseus* and *S. griseoflavus*, although the timing of onset of antibiotic production remained unchanged for both strains.

Role of SAM in antibiotic production.

In *S. coelicolor*, overexpression of SAM synthetase stimulated expression of the pathway-specific regulatory gene *actII-ORF4*⁵. To investigate the role of SAM in

Fig. 2. Effect of SAM on bicozamycin production in *S. griseoflavus*.

Wild-type strain of *S. griseoflavus* (FERM1805) was grown in a synthetic medium as previously described⁹. SAM (1 mM) was added at the time of inoculation (○) or at 24 hr (□). The closed symbol (●) represents no addition of SAM. Bicozamycin was determined by a bioassay with *Escherichia coli* strain BS-10 (our stock) as a test organism⁹.

streptomycin production, we carried out an RNase protection assay for *strR* transcription, since *strR* gene is known to be a pathway-specific positive regulatory gene for streptomycin biosynthesis^{10, 11}. The transcript for *strR* was detected in the cells in the transition (3 days) and stationary (5 days) phase (Fig. 3). As expected, we found a 1.35-fold increase in *strR* transcript in the cells grown with SAM for 3 days (verified by radio activity measurement), although virtually no difference was detected between the cells grown for 5 days. In agreement with the results from *strR* transcript analysis, the cells grown for 3 days with 1 mM SAM demonstrated a 1.9-fold greater activity of streptomycin kinase, an enzyme for streptomycin biosynthesis, over the cells grown without SAM (Table 1).

There are several reports that indicate the possible involvement of intracellular SAM in the regulation of antibiotic production^{12–15}. Recently, Kim *et al.*¹³ showed that methionine repressed the synthesis of a methyltransferase, which catalyzes the conversion of demethylrapamycin to rapamycin, along with suppressing the SAM synthetase itself. The repressive effect of methionine on the production of other antibiotics may involve a similar mechanism. However, since the bicozamycin biosynthetic pathway contains no steps requiring SAM as a methyl

Fig. 3. RNA protection assay of *strR* transcription.

The *Streptomyces griseus* strain IFO13189 was grown in a synthetic medium with or without 1 mM SAM (added at the time of inoculation) as shown in the Fig. 1. RNA was isolated from cells grown for 3 or 5 days using an ISOGEN reagent (Nippon Gene) and subjected to RNase protection assay. A 366-bp fragment containing a *strR* promoter region was generated by PCR using the primer RPA-*strR*-F (5'-CATGCGGACAGCTTTACTTGG-3') and RPA-*strR*-R (5'-TCGGTGCATGCCGTCGATGAC-3'). A T7 promoter sequence was added to the PCR fragment using a Lig'nScribe kit (Ambion, Austin, Texas), and in vitro transcription was carried out with a MAXIScript in vitro transcription kit (Ambion) and [α -³²P]UTP. Hybridizations and RNase digestion were carried out using a RPA III RNase protection assay kit (Ambion) according to the manufacturer's instructions. Total RNA (10 μ g) was used for each reaction. Protected fragments were analyzed on a 5% polyacrylamide / 8 M urea gel. An ethidium bromide-stained gel containing 0.5 μ g of RNA in each lane demonstrates the integrity of the RNA preparation.

Table 1. Changes in specific activity of streptomycin kinase of the cells grown in the presence or absence of SAM.

SAM added	Specific activity (U/mg of protein)		
	3 days	5 days	7 days
none	1.42	1.44	1.44
1 mM	2.64	1.89	1.44

Cells were grown in synthetic medium for the indicated times with or without SAM. Streptomycin kinase was assayed as described by Hara and Beppu¹⁶⁾. One unit of streptomycin kinase was defined as the amount that catalyzes the formation of 1 μ mol of inactivated streptomycin per hr. Mean values of two separate experiments are presented.

donor, the mechanism by which SAM affects bicozamycin production apparently differs from that for rapamycin.

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