ISSN 0026-2617, Microbiology, 2008, Vol. 77, No. 5, pp. 639–641. © Pleiades Publishing, Ltd., 2008.
Original Russian Text © D.G. Kozlov, V.S. Soifer, I.A. Malanicheva, O.V. Efremenkova, 2008, published in Mikrobiologiya, 2008, Vol. 77, No. 5, pp. 716–719.

SHORT COMMUNICATIONS

Search for Biosynthetic Precursors of A-Factor Group Regulators, Endogenous Regulators of Development of Actinomycetes

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Received July 23, 2007

DOI: 10.1134/S0026261708050226

Recently, a high emphasis has been placed on specific regulation of the processes of development in prokaryotes. A number of specific signal molecules of different chemical nature have been described, which are formed by microorganisms and affect such processes as growth, morphological differentiation, synthesis of secondary metabolites, state of dormancy, induction of cell growth and division, adaptation to stress, reactivation of "viable but nonculturable" cells, etc. [1–8]. One of the first described regulators of prokaryotes was A-factor, 2S-isocapryloyl-3S-hydroxymethyl-γ-butyrolactone, isolated from the actinomycete *Streptomyces griseus* (Fig. 1) [9]. Extension of the range of actinomycete species under study revealed that the regulators similar to A-factor are widespread among actinomycetes of various taxonomic groups [10–12]. Determination of the chemical structure of at least 15 regulators, which exhibited similar chemical composition and cross biological action, suggests the existence of a group of A-factor regulators in actinomycetes; they are supposedly synthesized via a similar mechanism [13–19]. It has been suggested that fatty acid residues and glycerol are the initial metabolites for the biosynthesis of A-factor group regulators [9]. This is indirectly confirmed by the fact that cerulenin (the antibiotic known as an inhibitor of fatty acid biosynthesis) also suppresses production of A-factor group regulators [20].

The goal of this work was to prove the assumption concerning the role of fatty acids in A-factor biogenesis.

The object of research was mutant strain *Streptomyces griseus* 1439 deficient in A-factor synthesis, which had been used as the main test culture in A-factor studies for 40 years. Under the action of A-factor present in the medium, the formation of aerial mycelium and spores in strain 1439 is recovered. Thus, the recovery of morphogenesis suggests the presence of A-factor, its close homologues, or analogs in the medium. Morphogenesis recovery becomes possible only under the influence of A-factor regulators [9, 21].

Strain 1439 was cultivated on a modified Gauze medium no. 2 containing the following (g/l): glucose, 1; peptone, 0.5; tryptone, 0.3; sodium chloride, 0.5; agar, 2; pH 7.2–7.4. The inoculum of strain 1439 was obtained by submerged cultivation for 2 days at 28°C on a shaker at 200 rpm in the medium containing the following (%): glucose, 4; soy meal, 3; sodium chloride, 0.25; $(NH_4)_2SO_4$, 0.6; KH_2PO_4, 0.05; chalk, 0.6; distilled water; pH 7.0.

The following fatty acid β-ketoesters (FAKE) were used as supposed precursors of A-factor synthesis: I, $(CH_3)_2CH(CH_2)_4COCH_2COOCH_3$ and II, $-(CH_3)_2CHCH_2COCH_2COOCH_3$. FAKE and A-factor were synthesized by V.S. Soifer at the Institute of Bioorganic Chemistry, Russian Academy of Sciences. The biological effect of the tested substances was assessed by the appearance of aerial mycelium in the test strain 1439. For this purpose, water suspensions of the tested substances were vigorously stirred in a shaker to obtain fine suspensions, which was then added to the melted agar in petri dishes. The inoculum was placed as a drop onto the agar medium surface. The plates were incubated at 28°C for 5 days. The presence

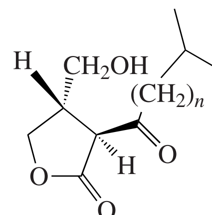

Fig. 1. The structure of autoregulators of *S. griseus* A-factor (n = 4) and its lower homologs: natural (n = 2 and 3) and artificially synthesized (n = 1).

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Induction of spore formation in the test strain *S. griseus* 1439, an indicator of A-factor

Analyzed substance	Concentration inducing spore formation, μg/ml
A-factor	10
$(CH_3)_2CH(CH_2)_4COCH_2COOCH_3$	500
$(CH_3)_2CHCH_2COCH_2COOCH_3$	5000

and intensity of aerial mycelium were assessed visually.

The used fatty acid β-ketoesters (FAKE) were shown to induce spore formation in the mutant 1439. In the control, the amount of A-factor inducing spore formation was 10 μg/ml, whereas 50 and 500 times higher content of FAKE I and FAKE II, respectively, were required to induce spore formation in strain 1439 (table). Therefore, the first of the above compounds may be considered a precursor of A-factor, while the second compound may be a precursor of its lowest artificial homologue. This conclusion is in agreement with the earlier observations showing that the biological activity of lower homologues is significantly less than the activity of A-factor, and the shorter the side chain of γ-butyrolactone, the lower the specific activity of the regulator [9].

The pathway of A-factor biosynthesis via fatty acids is also confirmed by the fact that the introduction of glycerol into an agarized medium in our experiments resulted in a two- to threefold decrease of the quantity of precursors (FAKE) required to induce the aerial mycelium formation. This fact can be explained by the better β-ketoester : glycerol molar ratio in the reaction of A-factor biosynthesis.

These findings support our hypothesis that A-factor biogenesis involves an *iso*-β-keto acid, which is cycled with participation of a molecule of oxidized glycerol with the formation of an unsaturated γ-lactone via the loss of water from the second position of oxidized glycerol, with subsequent reduction to A-factor (Fig. 2).

To date, the structures of 15 natural regulators (homologues or close analogues of A-factor) have been determined for the representatives of seven streptomycete species [13–19]. The common feature of the structure of these regulators is the presence of a γ-butyrolactone ring (butanolide), oxymethyl in position 3, and a fatty acid residue in position 2.

Autoregulators of some gram-negative bacteria (homoserine lactones) also contain γ-butyrolactone (butanolide) and a fatty acid residue. The absence of oxymethyl in position 3 and the presence of nitrogen are the main distinctive features of homoserine lactones as compared to the A-factor group regulators [9]. Note that the A-factor has no effect on the induction of secondary syntheses in the stationary phase (bioluminescence) in *Vibrio fisherii* and *Erwinia carotovora* [22]. Previously, our studies have shown that homoserine lactone formed by *V. fisherii* does not induce spore formation in the test strain for A-factor, *S. griseus* 1439. It is known that the initial metabolite in the biosynthesis of homoserine lactones is S-adenosyl methionine [23]. According to the presented results, the biosynthesis of A-factor regulators, in contrast to homoserine lactones, proceeds by another mechanism, in which the initial metabolites are fatty acid residues and glycerol.

Thus, it has been shown for the first time that extracellular microbial autoregulators (A-factor and acylated homoserine lactones) are synthesized in prokaryotic cells via different pathways. The data obtained are important for interpretation of the differences between

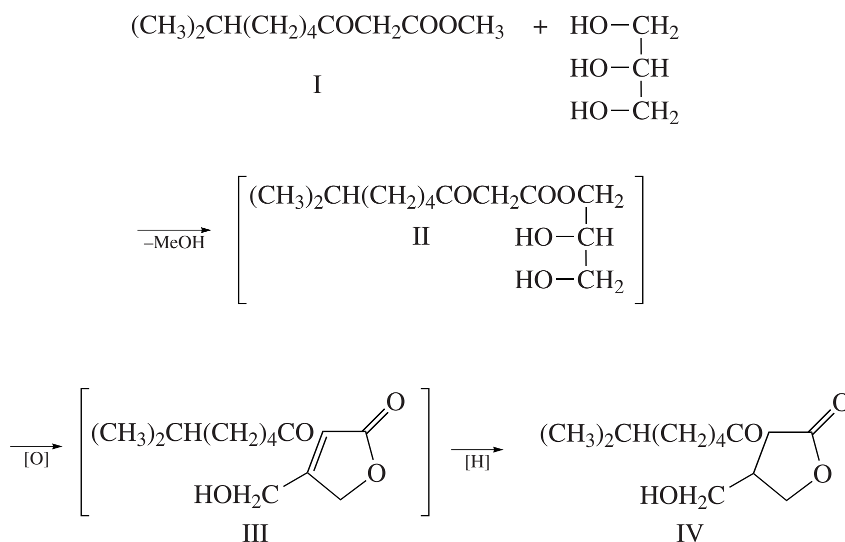

Fig. 2. The scheme of A-factor biosynthesis.

the functions of the two groups of low-molecular autoregulators possessing the common element, butanolide, in their structure.

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