EFFECT OF COBALT AND CYANOCOBALAMIN ON BIOSYNTHESIS OF A10255, A THIOPEPTIDE ANTIBIOTIC COMPLEX

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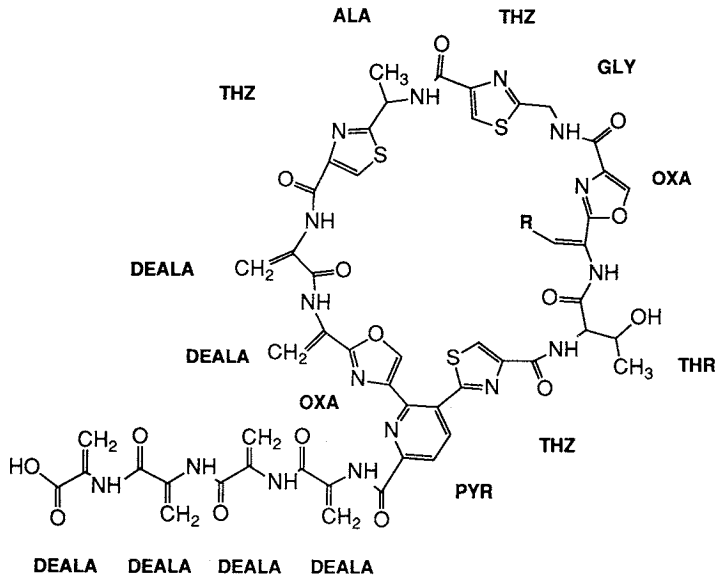
A10255, a thiopeptide antibiotic complex shown to promote growth and alleviate acidosis in ruminants, is produced by *Streptomyces gardneri*[1]. This complex, containing a cyclic peptide core with an amino acid side chain[2], is structurally similar to the known family of antibiotics whose members include berninamycin, nosiheptide, thiostrepton, sulfomycin I and thioxamycin[3~5]. The major factors of the complex are A10255G and A10255B (Fig. 1). These factors are identical except for the extent of methylation at R. Factor G contains a dehydrobutyrine residue (methyl group at R) while factor B contains a dehydronorvaline residue (ethyl group at R). A third factor, A10255E, contains a dehydronorleucine residue with an isopropyl group at R (M. DEBONO, personal communication). Initial fermentations in stirred reactors with a complex medium produced approximately 85% factor B and 15% factor G[1]. Subsequent fermentations in stirred reactors with a chemically defined medium produced 5% factor B and 95% factor G[6]. This report describes studies employing shaken flask cultures to investigate the A10255 factor ratio reversal observed when a defined medium was substituted for the complex medium in stirred reactors.

Mycelial stock cultures of NRRL 18260 were maintained in the vapor phase of liquid nitrogen. Seed cultures were grown in 250 ml wide-mouth Erlenmeyer flasks containing a medium consisting of glucose 0.75%, glycerol 0.5%, Trypticase soy broth 3%, enzyme-hydrolyzed casein 0.5%, potato dextrin 1% and $CaCO_3$ 1% in deionized water, pH

Fig. 1. Structure of A10255.



ALA, alanine; DEALA, dehydroalanine; GLY, glycine; OXA, oxazole; PYR, pyridine; THR, threonine; THZ, thiazole.

Factor	R=	Residue
A10255G	CH_3-	Dehydrobutyrine
A10255B	CH_3CH_2-	Dehydronorvaline
A10255E	$(CH_3)_2CH-$	Dehydroleucine

7.0 before autoclaving. The flasks were inoculated from liquid nitrogen stocks (1%) and incubated 72 hours at 30°C on a gyratory shaker orbiting at 250 rpm in a 5.08 cm diameter circle. Fermentation flasks and incubation conditions were as described above, except that 2% inoculum was used and the incubation period was 5~7 days. The complex (GGCM) fermentation medium contained glucose 1%, glycerol 3%, hydrolyzed casein (Sheffield Products) 1%, molasses 4%, NaH_2PO_4 0.01% and $CaCO_3$ 0.5%. The defined (GGA) fermentation medium contained glucose 0.5%, glycerol 3%, Na_2SO_4 0.2%, NH_4Cl 0.43%, $FeCl_3$ 0.0062%, $ZnCl_2$ 0.0019%, $MgCl_2 \cdot 6H_2O$ 0.03%, KH_2PO_4 0.1% and $CaCO_3$ 3.0%. A10255 was extracted from the cell mass with aqueous methanol and assayed by isocratic HPLC[1].

Shaken flask fermentations of A10255 with the complex (GGCM) medium yielded 285 μg/ml total A10255 consisting primarily of factor B (49%) with lesser amounts of factor G (41%) and minor amounts of factor E (10%). Fermentations in the defined (GGA) medium produced 550 μg/ml total A10255 containing predominantly factor G (86%) with factor B (14%) as a minor component. Because nutrition appeared to be responsible for this factor ratio reversal, media studies were conducted to determine which component of the GGCM medium supported biosynthesis of factors B and E. Supplementing the GGA medium with either molasses or molasses ash significantly increased the ratio of factors B and E to factor G (BE/G). In comparison to the unsupplemented GGA fermentations, addition of molasses increased the BE/G ratio 21-fold and addition of molasses ash increased the BE/G ratio 63-fold. These results suggested that the GGA medium was deficient in a trace metal essential for the biosynthesis of factors B and E. Eleven different minerals were then tested individually for their effect on the biosynthesis of A10255. Of the mineral salts present as standard components of the GGA medium, only higher concentrations of $FeCl_3$ increased the BE/G ratio. At a concentration of 1.52 mM, $FeCl_3$ increased the BE/G ratio 13-fold over the control medium (*i.e.*, 0.38 mM $FeCl_3$). Substitution of 1.52 mM $FeCl_2$ for the $FeCl_3$ increased the BE/G ratio three-fold over the control medium. Of the mineral salts tested as supplements to the GGA medium, only $CoCl_2$ substantially increased the BE/G ratio. At a concentration of 2.0 μM, $CoCl_2$ increased the BE/G ratio 56-fold over the unsupplemented medium. Lower levels of cobalt also increased the BE/G ratio (Table 1). Cobalt is an essential component of cobalamin (vitamin B_{12}), a coenzyme involved in methylation and rearrangement reactions[7]. Therefore, the effect of cobalamin supplementation on methylated factor biosynthesis was tested. As with cobalt, supplementing the GGA medium with low concentrations of cyanocobalamin significantly increased the BE/G ratio (Table 2). Further, supplementation of the GGA medium with equivalent molar concentrations of either cobalt or cyanocobalamin produced similar factor ratios. A similar effect has been reported for the methylated antibiotics mitomycin[8], fortimicin A[9] and coumermycin A_1[10], whose levels were increased by addition of either cobalt or cobalamin to the fermentation.

Table 1. Effect of cobalt supplementation on A10255 factor biosynthesis in GGA medium.

Cobalt addition (μM)[a]	Factor			BE/G[b]	Total A10255 (% of control)[c]
	G	B	E		
None[c]	86[d]	14	0	0.16	100
0.01	68	37	1	0.56	103
0.5	9	62	29	10.1	74
2.0	10	62	28	9.0	74
10.0	7	57	36	13.2	50

[a] Chloride salt, filter-sterilized.
[b] Ratio of factors B and E to factor G.
[c] Control, unmodified GGA medium.
[d] Percent of total.

Table 2. Effect of cyanocobalamin on A10255 factor biosynthesis in GGA medium.

Cyanocobalamin addition (μM)[a]	Factor			BE/G[b]	Total A10255 (% of control)[c]
	G	B	E		
None[c]	86[d]	14	0	0.16	100
0.01	65	34	1	0.54	103
0.5	9	65	26	10.1	112
2.0	7	61	32	13.3	95
10.0	5	63	32	19.0	88

[a] Filter-sterilized.
[b] Ratio of factors B and E to factor G.
[c] Control, unmodified GGA medium.
[d] Percent of total.

The stimulation of factors B and E biosynthesis by iron was apparently due to cobalt present as an impurity. Quantitative analyses by atomic absorption spectroscopy indicated that ferric chloride contained 0.019 μg cobalt/mg while ferrous chloride contained 0.004 μg cobalt/mg. These levels of cobalt were adequate for stimulation of factors B and E

biosynthesis. The dissimilar degree of stimulation demonstrated by the two forms of iron was apparently due to differences in the amount of cobalt each contained as an impurity. Enhancement of biosynthesis due to an impurity was reported by CLARIDGE *et al.*, who attributed stimulation of coumermycin A_1 synthesis by nickel to small amounts of cobalt present in the nickel[10].

Cobalamin is a coenzyme for the methyltransferase mediating the terminal step in the biosynthesis of methionine, a common donor of methyl groups *via* *S*-adenosylmethionine[11]. The effect demonstrated by addition of cobalt or cobalamin to the GGA medium was postulated to be due to increased synthesis of methionine, which then increased the BE/G ratio by functioning as a methyl donor for factor G. Addition of methionine (10 mM) to the GGA medium resulted in a small increase in the percentage of factors B and E (BE/G ratio = 0.30), much less than that resulting from cobalt or cobalamin supplementation. Methionine addition to the GGA medium supplemented with cobalt did not alter the factor ratio, although total synthesis was decreased. However, incorporation studies with L-[*methyl*-^{13}C]methionine have demonstrated that methionine is a methyl donor for the additional methyl groups of factors B and E[12].

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