VALINE AS A PRECURSOR OF *n*-BUTYRATE UNIT IN THE BIOSYNTHESIS OF MACROLIDE AGLYCONE

Sir:

The biosynthesis of macrolide antibiotics is inhibited by ammonium ions.[1] Previous papers[1~3] from this laboratory reported that when producing organisms were grown in the presence of ammonium ion-trapping agents such as magnesium phosphate, the inhibitory effect of ammonium ion was reduced and macrolide production increased. In an attempt to clarify the mechanism of the effect of ammonium ion, we studied the correlation between amino acid metabolism and macrolide biosynthesis. The present communication describes that valine catabolism plays an important role in supplying both *n*-butyrate and propionate units for the biosynthesis of aglycones of the 16-membered macrolide antibiotics, leucomycin and tylosin.

Preliminary results showed that the addition of valine or leucine increased the production of leucomycin, tylosin and protylonolide in *Streptoverticillium kitasatoensis* KA-468 (68-69-1), *Streptomyces fradiae* KA-427 and its mutant, strain KA-427-261, respectively. Since protylonolide is a biosynthetic intermediate with a lactonic structure corresponding to the aglycone moiety of tylosin,[4] these increases in production imply that valine serves as a precursor or a regulatory effector for the biosynthesis of these aglycones. This assumption led us to examine if the carbons of valine were incorporated into the carbon skeleton of aglycones of the antibiotics.

The ^{13}C NMR spectrum of leucomycin A_5 labeled by [2-^{13}C]valine with *S. kitasatoensis* KA-468 revealed that carbon 5 of the aglycone moiety and carbon 8″ of the *n*-butyryl moiety, which should be derived from the carboxyl carbon atom of *n*-butyrate,[5] were enriched to the extent of 5.0 and 1.6 times, respectively, in comparison with natural abundance with virtually no enrichment on the other carbons.

Valine is known to be metabolized to methylmalonyl-CoA *via iso*-butyrate in various biological systems.[6] The above incorporation profile therefore suggests that valine was transformed into a *n*-butyrate unit through a hitherto unknown pathway, in which *iso*-butyrate is a possible intermediate, as shown in Scheme 1-A. In order to confirm this suggestion, feeding experiments were performed using chemically synthesized [3,3′-$^{13}C_2$]*iso*-butyrate.

S. kitasatoensis KA-468 and *S. fradiae* KA-427-261 were grown in leucomycin[5] and protylonolide[4] production media, respectively, at 27°C for a total of 114 hours. The ^{13}C-labeled precursor was fed to the media at the 24th hour of cultivation. The ^{13}C-labeled leucomycin A_5 and protylonolide were isolated by a standard work-up described previously[5] and purified by preparative thin-layer chromatography on silica gel.

The incorporation of [3,3′-$^{13}C_2$]*iso*-butyrate into the leucomycin A_5 and protylonolide molecules is summarized in Table 1 and Fig. 1. Previous biosynthetic studies[5] indicated that carbons 5, 6, 17 and 18 of leucomycin, and carbons 5, 6, 19 and 20 of tylosin are derived from butyrate. The methyl carbon atoms of *iso*-butyrate were incorporated into carbons 6, 18 and 9″, 11″ of leucomycin A_5 with enrichment factors of 15.9~16.4 and 3.1~3.9, respectively. Carbons 6 and 20 of protylonolide similarly were enriched to the extent of 3.7~5.5

Scheme 1. Transformation of [2-^{13}C]valine and [3,3′-$^{13}C_2$]*iso*-butyrate to *n*-butyrate and propionate in the biosynthesis of leucomycin and protylonolide.

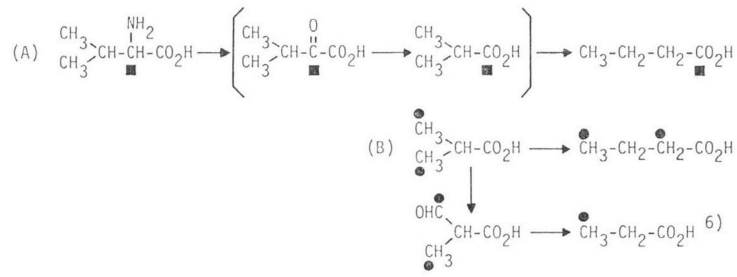

Fig. 1. Incorporation of ^{13}C-labeled precursors into leucomycin A_5 and protylonolide.

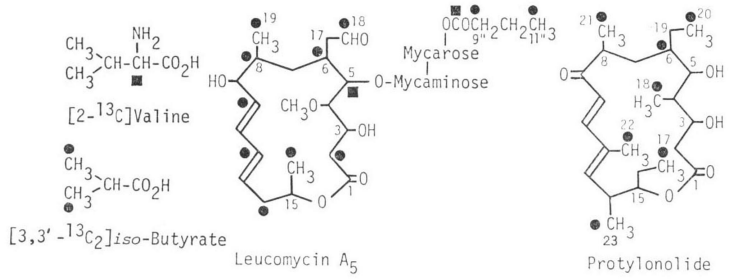

Table 1. Incorporation of [3,3'-$^{13}C_2$]*iso*-butyrate.

Carbon atom	Enrichment factor[a]		Carbon atom	Enrichment factor[a]	
	Leucomycin A_5	Protylonolide		Leucomycin A_5	Protylonolide
C- 1	0.8	0.8	C-14	5.8	1.0
C- 2	5.8	1.1	C-15	1.1	1.1
C- 3	0.7	1.1	C-16	4.4	1.1
C- 4	1.1	1.1	C-17	0.8	4.3
C- 5	0.7	0.8	C-18	16.4	4.0
C- 6	15.9	3.7	C-19	6.3	0.8
C- 7	1.1	1.0	C-20	1.2	5.5
C- 8	3.3	1.0	C-21		3.2
C- 9	1.2	0.9	C-22		4.2
C-10	4.7	1.2	C-23		4.0
C-11	1.2	1.1	C- 9″	3.1	
C-12	5.9	0.9	C-11″	3.9	
C-13	1.2	1.1			

[a]; Peak height $\frac{\text{enriched sample}}{\text{natural abundance}}$ from spectra run under essentially identical conditions.

times. These labeling patterns are in agreement with the assumption that valine is transformed to a *n*-butyrate unit *via* the migration of the carboxyl group of *iso*-butyrate, as shown in Scheme 1-B. In addition, the labeling of carbons 2, 10, 12, 14 and 16 of leucomycin A_5, which originate from the methyl carbon atom of acetate,[5] due to the metabolism of *n*-butyrate to acetate by β-oxidation, offers further support for the isomerization of *iso*-butyrate to *n*-butyrate. The enrichments of carbons 8 and 19 of leucomycin A_5 would be accounted for by the metabolism of [2-^{13}C]acetate to [2,3-$^{13}C_2$]propionate *via* the glyoxylate cycle.[7] The enrichments of carbons 17, 18, 21, 22 and 23 in protylonolide and carbon 19 in leucomycin A_5, which arise from methyl carbon atom of propionate,[5] can be explained on the basis of the known metabolic pathway:[6] [3,3'-$^{13}C_2$]*iso*-butyrate → [3,4-$^{13}C_2$]-methylmalonic acid semialdehyde → [3-^{13}C]propionate. Thus, we propose that the carbons of valine are incorporated into leucomycin A_5 and protylonolide as depicted in Fig. 1 in *S. kitasatoensis* KA-468 and *S. fradiae* KA-427-261, respectively.

Several papers[8~13] have suggested amino acid origins for the 4″-acyl side chains on the mycarosyl moiety of 16-membered macrolide antibiotics. However, the origins of the *n*-butyrate unit for aglycone biosynthesis have not been discussed. VANÉK *et al.*[14] suggested the possible correlation between valine catabolism and *n*-butyrate with regard to the biogenesis of the polyether antibiotic monensin.[14] Our experiments unequivocally demonstrate that several carbons of the aglycone moiety of 16-membered macrolides can originate from valine, and that the new valine metabolism may involve the iso-

merization of *iso*-butyrate to *n*-butyrate by the migration of the carboxyl group.

For further confirmation, experiments are in progress for the enzymatic and stereochemical characterization of the metabolism of valine to *n*-butyrate.

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