BIOSYNTHETIC SIMILARITY BETWEEN *STREPTOMYCES TENJIMARIENSIS* AND *MICROMONOSPORA OLIVASTEROSPORA* WHICH PRODUCE FORTIMICIN-GROUP ANTIBIOTICS

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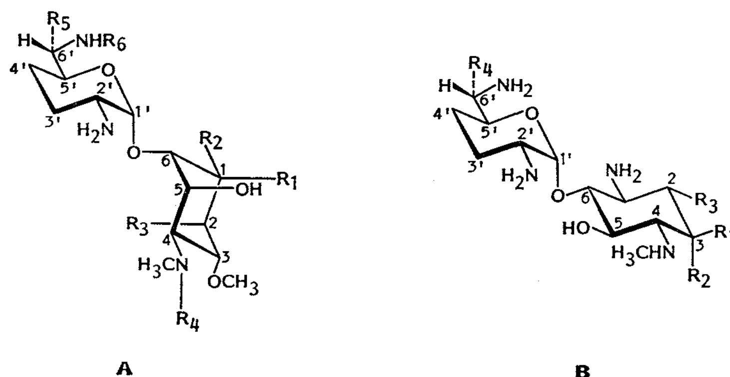
(Received for publication November 16, 1988)

The profile of bioconversion products of istamycin (IS) components by a blocked IS mutant of *Streptomyces tenjimariensis* that lost IS-productivity suggested a possible biosynthetic pathway of IS similar to that of fortimicin (FT) by *Micromonospora olivasterospora*. Both organisms are resistant to the antibiotics produced by each other. Based on these similarities, they were examined for their capability to convert an FT-intermediate (FT-B) and IS-intermediates (IS-A_0 and -B_0) through their biosynthetic pathways. *S. tenjimariensis* formed 1-*epi*-FT-B, 2″-*N*-formimidoyl-FT-A (=dactimicin) and 1-epidactimicin (a new antibiotic) from FT-B. On the other hand, *M. olivasterospora* converted IS-A_0 and -B_0 to 2″-*N*-formimidoyl-IS-A (=IS-A_3) and -B (=IS-B_3), respectively. Thus, the similarity in antibiotic biosynthesis was confirmed between these FT-group antibiotic-producing organisms. It was also found that the major fermentation product of *M. olivasterospora* is not FT-A (astromicin) but dactimicin.

By taking advantage of low substrate-specificity of enzymes involved in antibiotic biosynthesis, directed conversion or incorporation of specific compounds to new antibiotics has been successfully attempted in various actinomycete strains that produce antibiotics[1~3]. One such attempt was the biosynthetic conversion of foreign antibiotics (kanamycins; KM's) to new antibiotics (combimicins) with a gentamicin (GM)-producing *Micromonospora* strain[4]. This conversion by the GM-biosynthetic enzymes was attempted based on the structural similarity between KM and a GM-intermediate so that KM might be transformed by GM-biosynthetic enzymes. Resistance to KM by the GM-producing *Micromonospora* is probably a key point since it seems unlikely that the organism is capable of metabolizing KM if it is sensitive to KM. GM-producing strains of *Micromonospora* have been known to be resistant to KM[5,6].

Istamycins (IS's) are produced by *Streptomyces tenjimariensis*[7,8] and share a pseudodisaccharide moiety with fortimicin (FT) produced by *Micromonospora olivasterospora*[9~11] (Fig. 1). This structural similarities suggest a similarity in the antibiotic biosynthetic pathways and a possible resistance to each antibiotic by the producers. We attempted the conversion of intermediates of both IS and FT by *M. olivasterospora* and *S. tenjimariensis*. It was our special interest whether *S. tenjimariensis* could form a new antibiotic (1-epidactimicin; EDC) from FT components because only this organism has been known to produce epimers in terms of NH_2 group at C-1 position as shown in Fig. 1. As expected, bioconversion experiments verified the biosynthetic similarity between these two organisms and resulted in the formation of EDC from FT-B by *S. tenjimariensis*. It was also found

Fig. 1. Structures of antibiotics studied.



Basic structure	Antibiotic	R_1	R_2	R_3	R_4	R_5	R_6
A	IS-A_0	NH_2	H	H	H	H	CH_3
	IS-A	NH_2	H	H	$COCH_2NH_2$	H	CH_3
	IS-A_3	NH_2	H	H	$COCH_2NHCH{=}NH$	H	CH_3
	IS-B_0	H	NH_2	H	H	H	CH_3
	IS-B	H	NH_2	H	$COCH_2NH_2$	H	CH_3
	IS-B_3	H	NH_2	H	$COCH_2NHCH{=}NH$	H	CH_3
	FT-A	NH_2	H	OH	$COCH_2NH_2$	CH_3	H
	FT-B	NH_2	H	OH	H	CH_3	H
	1-*epi*-FT-B	H	NH_2	OH	H	CH_3	H
	DC	NH_2	H	OH	$COCH_2NHCH{=}NH$	CH_3	H
	EDC	H	NH_2	OH	$COCH_2NHCH{=}NH$	CH_3	H
B	IS-Y_0	OCH_3	H	H	H		
	IS-X_0	H	OCH_3	H	H		
	FT-KH	OCH_3	H	OH	CH_3		
	FT-KR	H	OCH_3	OH	CH_3		

IS: Istamycin, FT: fortimicin, DC: dactimicin, EDC: 1-epidactimicin.

that the major fermentation product of *M. olivasterospora* was not FT-A (astromicin) but dactimicin (DC) which was first reported with *Dactylosporangium matsuzakiense*[12,13].

Materials and Methods

Strains Used

S. tenjimariensis U41 is a blocked mutant of IS-biosynthesis obtained by UV-irradiation of IS-producing strain SS-1507. This strain is able to produce a small amount of IS-Y_0 and -X_0 but no detectable amount of other IS components such as IS-A_3 and -B_3 known as major fermentation products of the parental strain SS-1507. The strains U41 and FT-producing *M. olivasterospora* ATCC 21819 were used for conversion experiments.

IS's and FT's

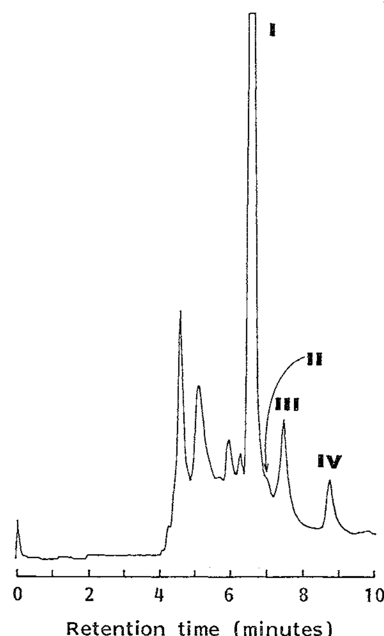
IS's (Fig. 1) were obtained from Dr. S. Kondo, Institute of Microbial Chemistry. FT's and DC were prepared from the cultured broth of *M. olivasterospora* as described elsewhere[14].

Conversion of IS's and FT-B

For conversion of IS components and FT-B, *S. tenjimariensis* U41 was first cultivated with rotary shaking (180 rpm) at 27°C for 3 days in a liquid medium (100 ml; pH 7.0) consisting of the following; corn gluten meal 5.0%, wheat germ 2.0%, $CaCO_3$ 0.6% and $MgSO_4 \cdot 7H_2O$ 0.05%. This culture

(1 ml) was transferred into an IS fermentation medium (100 ml; pH 7.0) consisting of wheat germ 6.5%, soy bean oil 3.5%, sodium palmitate 0.05%, $CaCO_3$ 0.6% and $MgSO_4 \cdot 7H_2O$ 0.05%. After incubation at 27°C for 3 days with rotary shaking, an IS component (Y_0, X_0, A_0, B_0, A, B, A_3 or B_3) or FT-B was added to the culture at 200 μg/ml. The incubation was continued for 7 more days. In case of *M. olivasterospora*, strain ATCC 21819 was shake-cultured at 27°C for 3 days in a medium (100 ml; pH 7.0) consisting of potato starch 2.0%, soy bean meal 2.0%, $MgSO_4 \cdot 7H_2O$ 0.1%, NaCl 0.3% and KH_2PO_4 0.1%. This seed culture (1 ml) was transferred into an FT fermentation medium (100 ml; pH 7.0) consisting of dextrin 5.0%, soy bean meal 3.5%, soy bean oil 1.0%, KH_2PO_4 0.3%, $Na_2HPO_4 \cdot 12H_2O$ 0.7%, $CaCO_3$ 0.7% and $CoCl_2$ 0.25×10^{-4}% and incubated on a rotary shaker (180 rpm) at 27°C. For conversion of IS's by the strain ATCC 21819, IS-A_0 or IS-B_0 at 200 μg/ml was added after 3 days of fermentation and the incubation was continued at 27°C for 2 or 3 days.

Fig. 2. HPLC chromatogram of conversion products from fortimicin B (FT-B) by *Streptomyces tenjimariensis* U41.



Retention times of peaks I, II, III and IV were identical with those of FT-B, 1-*epi*-FT-B, dactimicin and 1-epidactimicin, respectively.

Analysis of Products of Conversion and Fermentation

Cultured broths were adjusted to pH 2 with HCl and filtered. The resultant filtrate was neutralized (pH 6.0) with NaOH and passed through a column of Amberlite IRC-50 (Na^+ - H^+, 7:3). The adsorbed IS or FT compounds were eluted with 0.5 N H_2SO_4. After adjusted to pH 5.5 and concentrated, the resulting precipitate was removed from the eluate. Then the eluate was analyzed with a reverse-phase HPLC (Fig. 2) using a column of YMC gel ODS-5 μ (Yamamura Chemical Co., Japan) and an eluant, 4% aqueous acetonitril, containing 0.02 M sodium-1-pentasulfonate, 0.2 M sodium sulfate and 0.1% acetic acid. Antibiotics eluted were monitored by UV absorption at 344 nm following *o*-phtalaldehyde reaction of the eluate in boric acid (pH 10). Structures of the products were further analyzed by NMR using Jeol JNM-GX400, if appropriate[14].

Results

Conversion of IS's by *S. tenjimariensis*

The IS components produced by *S. tenjimariensis* SS-1507 were tested for conversion by the IS-nonproducing mutant, U41 (Table 1). Conversion products, IS-A_3, -B_3, -A_0 and -B_0 were detected from almost all substrates except for IS-A and -B. Formation of these four compounds from each other suggested a sort of biosynthetic network among these compounds. By contrast, IS-Y_0 was not formed from any other IS compound as substrate. Instead, it was converted to all the IS compounds but IS-A and -B. IS-X_0 was formed only from IS-Y_0 and converted clearly to IS-A_3, -B_3, -A_0 and -B_0. On the other hand, a small amount of IS-A and -B were detected in almost all cultured broths. However, their formation seemed to be due to chemical decomposition by elevated pH of the cultured broth, but not to enzymatic reaction, because IS-A_3 and -B_3 are known to be easily

Table 1. Conversion products of istamycins by *Streptomyces tenjimariensis*.

Substrate (istamycins)	Conversion product (istamycins)							
	Y_0	X_0	A_0	B_0	A_3	B_3	A	B
Y_0		+++	+++	+++	+++	+++	+	+
X_0	—		+++	+++	+++	+++	+	+
A_0	—	—		+++	+++	+++	+	+
B_0	—	—	±		±	+++	+	+
A_3	—	—	+++	+++		+++	+	+
B_3	—	—	±	+++	+++		+	+
A	—	—	—	—	—	—		—
B	—	—	—	—	—	—	—	

Conversion of istamycin components was expressed with $+\sim+++$ according to the relative amount of conversion products formed. ±: Variable detection, —: no formation.

Table 2. Ratio of A type istamycins to B type istamycins in the fermentation broth of *Streptomyces tenjimariensis*.

Incubation temperature	11-Day incubation			14-Day incubation		
	A	B	B/A	A	B	B/A
27°C	1.0	0.8	0.8	1.2	0.7	0.6
30°C	1.3	2.6	2.0	1.6	2.6	1.6

Relative values of A and B types of istamycin to A type istamycins (1.0) accumulated by 11-day incubation were scored.

decomposed to IS-A and -B in alkaline pH[8].

In relation to conversion to epimeric compounds (A and B types) in terms of NH_2 group at C-1 position, it was found that accumulation ratio of these epimers in fermentation broth of IS-producing strain SS-1507 varied depending on incubation temperature. As shown in Table 2, accumulation of A type was higher than that of B type when incubated at 27°C and *vice versa* at 32°C.

Conversion of Intermediates of Foreign Antibiotics

The conversion experiments of IS components suggested that the biosynthetic pathway of IS was very similar to that of FT. Therefore, it seemed likely that intermediates of IS and FT could be converted to antibiotic substances by the FT (*M. olivasterospora*) and IS (*S. tenjimariensis*) producers. Both producers were then examined for their resistance to FT-A and IS-A which seemed to be another key factor for this conversion. These organisms showed a high resistance (at least 200 μg/ml) to both IS-A and FT-A in fermentation media.

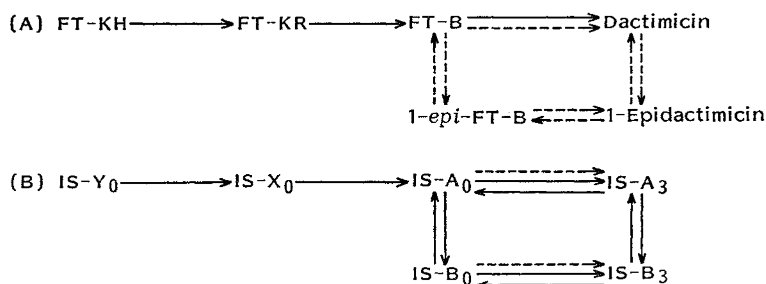
Another point to note in this attempt was that formation of EDC from FT-B could be expected by the epimerase activity of *S. tenjimariensis* if FT-B was transformed through IS-biosynthetic pathway. Bioconversion results are summarized in Table 3. *S. tenjimariensis* U41 formed DC and EDC from FT-B as expected. On the other hand, *M. olivasterospora* accumulated only IS-A_3 and IS-B_3 from IS-A_0 and IS-B_0, respectively, in a detectable amount as expected. No detectable IS-A or IS-B was accumulated. The accumulation of formimidoylated-IS compounds (IS-A_3 and -B_3) was unexpected since the reported biosynthetic pathway of FT[15,16] did not involve any formimidoylated compounds. Then, we analyzed the fermentation products of *M. olivasterospora*. It turned out that only FT-B and DC and not FT-A were detected (Table 3). Similarly, IS-producing *S. tenjimariensis* SS-1507 did not accumulate IS-A and -B but IS-A_3, -B_3, -A_0 and -B_0 as we reported previously[8].

Table 3. Products of conversion and fermentation by *Streptomyces tenjimariensis* and *Micromonospora olivasterospora*.

Product	*S. tenjimariensis*		*M. olivasterospora*		
	Conversion FT-B	Fermentation	Conversion		Fermentation
			IS-A_0	IS-B_0	
IS-A_0	−	++	/	−	−
IS-B_0	−	+	−	/	−
IS-A_3	−	+++	+	−	−
IS-B_3	−	++	−	+	−
FT-A	−	−	−	−	−
FT-B	/	−	−	−	++
1-*epi*-FT-B	+	−	−	−	−
DC	+	−	−	−	+++
EDC	+	−	−	−	−

Formation of products was expressed with + ~ +++ according to the relative amount of production.
−: No production.
IS: Istamycin, FT: fortimicin, DC: dactimicin, EDC: 1-epidactimicin.

Fig. 3. Possible biosynthetic pathways of fortimicins and istamycins.



(A) Fortimicin (FT) biosynthesis by *Micromonospora olivasterospora*. Dotted line: Conversion by *Streptomyces tenjimariensis*.
(B) Istamycin (IS) biosynthesis by *S. tenjimariensis*. Dotted line: Conversion by *M. olivasterospora*.

Discussion

Conversion experiments of IS components by *S. tenjimariensis* suggested a possible pathway of the later steps of IS biosynthesis. As shown in Fig. 3, the pathway from IS-Y_0 to IS-A_3 is very similar to that of FT-biosynthesis[15,16]. However, the pathway does not involve IS-A and -B, although they were detected in a small amount. Formation of these two substances could be due to chemical degradation of IS-A_3 and -B_3 probably by a relatively long exposure to the elevated pH of the cultured broth. In the early stage of our study on the isolation of fermentation products of *S. tenjimariensis*, we reported that IS-A and -B were the major products[7]. However, we found later that IS-A and -B were not metabolic products but chemical degradation products of true major fermentation products (IS-A_3 and -B_3)[8]. Both IS-A_3 and -B_3 are so unstable in alkaline solution that IS-A, -B, -A_1, -B_1, -A_2 and -B_2 can be detected if alkaline solution such as ammonium hydroxide is used for elution of fermentation products adsorbed to cation exchange resin. Thus, minimizing exposure of IS fermentation products to alkaline pH resulted in the exclusive detection of IS-A_3, -B_3, -A_0 and -B_0 as shown in Table 3. This was also true in *M. olivasterospora* ATCC 21819. This strain did not form FT-A type compounds in both conversion of IS's (IS-A_0 and -B_0) and FT fermentation, but formimidoylated compounds; *i.e.* IS-A_3 and -B_3 by conversion of IS compounds and dactimicin (=2″-*N*-formimidoyl-FT-A) and FT-B by fermentation. Distinct difference in the antibiotic biosynthetic pathways of *S. tenjimariensis*

from *M. olivasterospora* is the production of epimers. Because of the epimerase activity of *S. tenjimariensis*, the final steps of IS-biosynthesis will form a kind of metabolic grid as shown in Fig. 3. Neither *M. olivasterospora* nor any other FT-group antibiotic producer has been known to produce epimers of their antibiotics. This fact was one of the basis for our speculation that a new antibiotic (EDC) would be formed from FT-B by *S. tenjimariensis*. Based on our study[17], the resistance mechanism of *S. tenjimariensis* to FT-group antibiotics seems to be dependent on ribosomal resistance and not to inactivating enzymes. This was another basis of our attempt. If the biochemical basis of the resistance was dependent upon an inactivating enzyme, conversion of foreign antibiotics might result in the formation of inactivated substances. The same situation can be seen in the GM-producing strain of *Micromonospora* which produces combimicins from KM's. The resistance mechanism of a GM-producing strain of *Micromonospora*[6] has been known to depend upon ribosomal resistance. It should be also noted that *S. tenjimariensis* failed to convert aminoglycoside antibiotics other than the FT-group to antibiotic compounds, although the organism was resistant to these antibiotics (unpublished data). Furthermore, the GM-producing *Micromonospora* strain, which was used for the production of combimicins from KM, failed to convert neamine which has a structural similarity to GM (unpublished data). This failure could be related with the sensitivity to neamine of the strain. In a deoxystreptamine-negative mutant of *Micromonospora inyoensis* which produces sisomicin, conversion of neamine to sisomicin has been reported[18]. However, the conversion rate from neamine was much lower than that from paromamine. This difference might also be due to the difference in the resistance to paromamine and neamine of the sisomicin producer. Thus, both structural similarity and resistance mechanisms as well as the substrate specificity of biosynthetic enzymes should be key factors for antibiotic-producing actinomycetes to convert foreign antibiotics to novel antibiotics.

Although successful, the conversion rate of FT-B to DC and EDC by *S. tenjimariensis* was as low as 1% as in the case of mutasynthesis or combimicins. This low conversion rate may be improved by establishing the suitable conditions for the conversion or by strain improvement. A promising strain improvement will be the construction by gene technology of FT-producing *M. olivasterospora* which contains genes directing the synthesis of IS-biosynthetic enzymes.

Acknowledgments

We are grateful to Dr. S. KONDO for istamycins and the Late Prof. H. UMEZAWA for encouragement.

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