Purification, Characterization, and Kinetic Mechanism of S-Adenosyl-L-methionine:Macrocin O-Methyltransferase from Streptomyces fradiae* 

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S-Adenosyl-L-methionine:macrocin O-methyltransferase catalyzes conversion of macrocin to tylosin, the terminal and main rate-limiting step of tylosin biosynthesis in Streptomyces fradiae. The O-methyltransferase was stabilized in vitro and purified to electrophoretic homogeneity. The purified enzyme had a molecular weight of 65,000 and consisted of two identical subunits of 32,000 with an isoelectric point of 4.5. The enzyme required Mg⁺⁺, Mn⁺⁺, or Co⁺⁺ for maximal activity and was catalytically optimal at pH 7.5–8.0 and 31 °C. The O-methyltransferase catalyzed the conversion of macrocin to tylosin at a stoichiometric ratio of 1:1. The enzyme also mediated conversion of lactenocin → desmycosin. The corresponding Vₘₐₓ/Kₐ ratios for the two analogous conversions were similar, and both enzyme conversions were susceptible to extensive competitive and noncompetitive inhibitions by macroclide metabolites. Steady-state kinetic studies for initial velocity, substrate analogue, and product inhibitions have allowed formulation of Ordered Bi Bi as the reaction mechanism for macrocin O-methyltransferase.

Tylosin, a 16-membered macrolide antibiotic produced by Streptomyces fradiae, is used in the swine industry as a growth promontant and in veterinary medicine for treatment of infections caused by Gram-positive bacteria and mycoplasma. The biosynthesis of tylosin and its general regulation have been of considerable scientific as well as industrial interest for over a decade (1, 2). Recent information on the biosynthetic pathway of tylosin (1, 3) makes it feasible to study regulation of tylosin biosynthesis at the enzyme level. Knowledge gained in such study may be applicable to tylosin yield improvement. We chose to examine S-adenosyl-L-methionine:macrocin O-methyltransferase initially since this enzyme catalyzes the terminal and major rate-limiting step (Fig. 1) of tylosin biosynthesis in S. fradiae (4, 5). Preliminary studies of macrocin O-methyltransferase from cell-free extracts of S. fradiae indicated that the specific activity of the enzyme was higher in mutants producing higher levels of tylosin (5) and that the enzyme was inhibited by tylosin and some of its precursors and shunt metabolites (6). However, the molecular nature of the rate-limiting reaction is still unknown. Plausible molecular mechanisms that are amenable to an enzymological investigation might be inadequate enzyme levels (5), metabolic inhibition (6), deficiency of cosubstrate (i.e. methyl group donor), or enzyme degradation. In this report, we describe an effective purification of macrocin O-methyltransferase to electrophoretic homogeneity. We show that the purified enzyme has a narrow substrate specificity and is subject to broad metabolic inhibitions by two distinctive kinetic patterns. In addition, our kinetic data strongly suggest that macrocin O-methyltransferase follows a compulsory ordered reaction mechanism.

EXPERIMENTAL PROCEDURES AND RESULTS

Substrate Specificity—In addition to macrocin, the O-methyltransferase was capable of catalyzing 3′-O-methylation of 20-dihydromacrocin, lactenocin, and 20-dihydrolactenocin (Table III) with AdoMet as the methyl-group donor. The enzyme activity with either form of macrocin was similar to that with either form of lactenocin. No enzymic methylation with demethylmacrocin, demethyl lactenocin, the four macroclide intermediates preceding demethylmacrocin in the preferred pathway (3), and tylactone was HPLC-detectable when each macrolide compound was examined for its disappearance and product formation under the optimal catalytic conditions of the enzyme. Among the six methyl-containing compounds tested (Table III), only AdoMet could serve as the methyl-group donor in macrocin O-methyltransferase-catalyzed methylation of macrocin or lactenocin.

Enzyme Inhibition—Macrocin O-methyltransferase-catalyzed conversion of macrocin to tylosin was inhibited moderately by demethyl lactenocin or demethyl macrocin and weakly by desmycosin or relomycin (Table IV). Similarly, macrocin O-methyltransferase-catalyzed conversion of lactenocin to desmycosin was inhibited moderately by demethyl lactenocin or demethyl macrocin and weakly by tylosin or relomycin. No substrate inhibition of the O-methyltransferase by macrocin or lactenocin was shown at a concentration up to 120 μM. Both macrocin O-methyltransferase-catalyzed conversions were inhibited weakly by AdoHcy and strongly by sinuefungin.

1 An initial work of this paper was presented (by W. K. Y.) in the 1984 Summer Gordon Research Conference, Meriden, New Hampshire, and two preliminary reports (abstracts K141 and K142) were presented (by N. J. B. and W. K. Y.) in the 1985 Annual Meeting of American Society for Microbiology, Las Vegas, NV.

2 Portions of this paper (including "Experimental Procedures," part of "Results," Figs. 2–9, and Tables 1 and 2) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

3 The abbreviations used are: AdoMet, S-adenosyl-L-methionine; MOMT, macrocin O-methyltransferase; PAE buffer, 15 mM KH₂PO₄, pH 7.5, containing 0.2 mM AdoMet and 10% (v/v) ethanol; AdoHcy, S-adenosyl-L-homocysteine; PMSF, phenylmethylsulfonyl fluoride; HPLC, high performance liquid chromatography; FPLC, fast protein liquid chromatography; and SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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FIG. 1. Macrocin O-methyltransferase-catalyzed conversion of macrocin to tylosin.

TABLE III

| Substrate specificity of macrocin O-methyltransferase | Compound tested | Relative activity |
|------------------------------------------------------|-----------------|-------------------|
|                                                      | Macrocin        | 100               |
|                                                      | 20-Dihydromacrocin | 86               |
|                                                      | Lactenocin      | 79                |
|                                                      | 20-Dihydrolactenocin | 84               |
|                                                      | Demethylmacrocin | 0                 |
|                                                      | Demethylactenocin | 0                 |
|                                                      | Methyl group containing\* | 100               |
|                                                      | AdoMet           | 100               |
|                                                      | $^a$-Methyltetrahydrofolate | 0               |
|                                                      | Betaine          | 0                 |
|                                                      | Choline          | 0                 |
|                                                      | Methylthioadenosine | 0               |
|                                                      | Methionine       | 0                 |

\*Tested at 40 $\mu$M with 250 $\mu$M AdoMet.
\textsuperscript{a}Tested at 250 $\mu$M with 49 $\mu$M macrocin.
\textsuperscript{a}No detectable formation of macrolide product by HPLC analysis.

or A9145C (Table V). AdoMet, the cosubstrate of either conversion, was not inhibitory at a concentration up to 1 mM.

DISCUSSION

Purification—Addition of phenylmethylsulfonyl fluoride, AdoMet, and ethanol to cell-free extracts of S. fradiae can protect macrocin O-methyltransferase from inactivation for a minimum of 1 week at 4 °C. Under the stabilizing conditions, the O-methyltransferase has been purified to apparent electrophoretic homogeneity by a simple four step chromatographic procedure (Table I). Protection of the O-methyltransferase from inactivation by phenylmethylsulfonyl fluoride and improvement of its stability by each of the four chromatographic steps indicate that enzyme lability is likely caused by a serine protease(s). Purification of macrocin O-methyltransferase, a tylosin biosynthetic enzyme from the preferred pathway (3), has facilitated molecular elucidation of the terminal and rate-limiting methylation at both enzyme and gene levels.

Success in isolation of biosynthetic enzymes from other macrolide pathways has been limited (2, 16).

Physical and Catalytic Properties—Macrocin O-methyltransferase has a molecular weight of 65,000 and a subunit size of 32,000; therefore, the native enzyme appears to be dimeric. Automated sequence analysis by Edman degradation of the purified enzyme revealed a single 84-residue amino-terminal sequence (17), suggesting that the O-methyltransferase consists of two identical subunits. The subunit identity is substantiated by the observation that an oligonucleotide probe constructed according to a part of the terminal sequence is specific for a single DNA sequence, which when cloned, restored the O-methyltransferase activity to a macrocin O-methyltransferase-negative mutant of S. fradiae (17, 18). Macrocin O-methyltransferase has no activity in the absence of a metal ion, and maximal activity can be shown with Mg\textsuperscript{2+}, Mn\textsuperscript{2+} or Co\textsuperscript{2+}. Possibly, an intrinsic divalent metal ion binds loosely to the O-methyltransferase in vivo and dissociates readily from the enzyme during the enzyme purification.

Substrate Specificity—The activity data (Table III) and kinetic constants (as described under “Initial Velocity Pattern” of the Miniprint) for the O-methyltransferase indicate that the enzyme catalyzes conversion of macrocin to tylosin and of lactenocin to desmycosin at an approximately equivalent efficiency. Such substrate specificity toward macrocin and lactenocin is in agreement with 3'-O-methylation of an attached 2'-O-methylated 6-deoxy-D-allose as indicated from previous biochemical studies of S. fradiae (3-5). Whether macrocin O-methyltransferase or an independent O-methyltransferase can catalyze 3'-O-methylation of free 2'-O-methylated 6-deoxy-D-allose as indicated from previous biochemical studies of S. fradiae (3-5). Whether macrocin O-methyltransferase or an independent O-methyltransferase can catalyze 3'-O-methylation of free 2'-O-methylated 6-deoxy-D-allose, presumed as a dTDP-derivative, remains to be determined. This enzymic methylation is implicated in formation of mycinose from another proposed route of tylosin biosynthesis (I.e. mycaminosyltylonolide +mycinose demycarosyltylosin +mycarose tylosin) in S. fradiae (1, 19). A possibility that 3'-O-methylations of macrocin and lactenocin are mediated by two separate O-methyltransferases was evaluated by an initial DEAE-Trisacryl...
nor demethylactenocin is a macrolide substrate for macrocin chromatography of a
where Hac-T-0 was 0.44 nmol/min and that as determined with 40
activity peak with macrocin as the macrolide substrate was 0.40 nmol/min. The designations for 12 → 15, 10 → 14, and ND are the same as those (nd) in Table IV.

Macrolide and Nucleoside Inhibitions—The range and extent of macrolide inhibitions for macrocin O-methyltransferase-catalyzed conversion of macrocin to tylosin are indistinguishable from those for macrocin O-methyltransferase-catalyzed conversion of lactenocin to desmycosin (Table IV). The inhibition of macrocin O-methyltransferase by tylosin, desmycosin, or relomycin (i.e. C-20 reduced form of tylosin) is characteristic of product inhibition (6), which has also been demonstrated for demethylmacrocin O-methyltransferase (20) and erythromycin C O-methyltransferase (21). The observation that both demethylmacrocin and demethylactenocin are the most potent macrolide inhibitors of macrocin O-methyltransferase (Tables IV and VI) is unique for an O-methyltransferase, since either metabolite of tylosin biosynthesis also serves as a macrolide substrate for demethylmacrolcin O-methyltransferase (20). A similar broad inhibition of macrocin O-methyltransferase from cell-free extracts of S. fradiae by macrolide compounds has been reported (4). We speculate that macrocin O-methyltransferase is subject to in vivo regulation by broad but selective metabolic inhibition rather than specific product inhibition. The structural requirement of a macrolide compound for inhibition of macrocin O-methyltransferase has not been defined; however, all five inhibitory macrolide compounds contain a mycosinosyltylactone core that is connected to 6-deoxy-d-allose or mycinosin (Table IV) as was pointed out previously (6). Presence of mycarosyl moiety or reduction at the C-20 position of tylosine has little effect on the enzyme inhibition.

The inhibition by AdoHcy and no inhibition by its d-isomer (Table V) indicates a high specificity of the nucleoside reaction toward macrocin O-methyltransferase. Sinefungin and A9145C are much more inhibitory than AdoHcy in macrocin O-methyltransferase-catalyzed methylation of rate demethylmacrocin O-methyltransferase. Therefore, the 2'-O-methylation of bound 6-deoxy-d-allose as catalyzed by demethylmacrocin O-methyltransferase is the prerequisite of the subsequent 3'-O-methylation by macrocin O-methyltransferase, and this methylation order is consistent with the isolation of two distinguishable O-methyltransferase-deficient mutants from S. fradiae as described previously (5, 6).

**Table IV**

| Macrolide Compound | Structural Representation | Inhibition (%) |
|--------------------|--------------------------|----------------|
| Tylosin(1)         | CH₃                      | nd, nd        |
| 23-Demethylmacrolin(3) | CH₃                    | nd, nd        |
| Lactenocin(10)     | HO-CHO                   | nd, nd        |
| Macrocin(12)       | HO-OH(2')                | 38, 39        |
| Desmycosin(14)     | HO-CHO                   | 14, 8         |
| Tylosin(13)        | HO-OH(2')                | 17, 11        |

*Macrolide compounds which were tested for inhibition of macrocin O-methyltransferase-catalyzed conversions include all nine metabolites (1-5, 8, 9, 12, 15) from the preferred pathway (3), two metabolites (10, 14) from the alternative methylation route (20), three shunt metabolites (6, 7, 16), and two C-20 reduced macroline metabolites (11, 13).

Each macrolide compound is represented in the following manner:

(3')HO OH(2');

(20)CH₃

where H₈C-T-O = tylosine, S₁ = mycosinose, S₂ = mycarose,

and (3')HO OH(2') = 6-deoxy-d-allose.

12 → 15 and 10 → 14 designate macrocin O-methyltransferase-catalyzed conversions of macrocin → tylosin and lactenocin → desmycosin, respectively. The uninhibitory activity of macrocin O-methyltransferase as determined with 40 µM macrocin and 250 µM AdoMet was 0.44 nmol/min and that as determined with 40 µM lactenocin and 250 µM AdoMet was 0.35 nmol/min. For inhibition analysis of the enzyme, each maclroline compound was added at 80 µM (i.e. at a total concentration of 120 µM when used as a macrolide substrate).

- nd, not detectable (i.e. ≤ 22% of either uninhibitory activity).
- -, not analyzed.

**Table V**

| Adenine-containing compound | Concentration | Inhibition (%) |
|-----------------------------|---------------|----------------|
|                             | µM            | 12 → 15, 10 → 14 |
| Adenine                    | 200           | ND             |
| 5'-Methylthiodenosine      | 200           | ND             |
| AdoMet                     | 1000          | ND             |
| AdoHcy                     | 50            | 36, 54         |
| 200                         | 75, 81        |
| d-Isomer of AdoHcy         | 200           | ND             |
| Sinefungin                 | 0.5           | 50, 31         |
| 2.0                        | 79, 56        |
| 10                          | 92, 78        |
| A9145C                     | 0.5           | 22, 37         |
| 2.0                        | 47, 45        |
| 10                          | 75, 82        |

*The uninhibitory activity of macrocin O-methyltransferase as determined with 56 µM macrocin and 200 µM AdoMet was 0.45 nmol/min and that as determined with 56 µM lactenocin and 200 µM AdoMet was 0.40 nmol/min. The designations for 12 → 15, 10 → 14, and ND are the same as those (nd) in Table IV.
macrocin or lactenocin (Tables V and VI). Thus, the structural selectivity of a nucleoside inhibitor appears to reside in the side chain rather than adenine or ribose moiety. The $K_m$ of sinefungin (i.e. 0.06 μM) is unusually low for a nucleoside inhibitor of a methyltransferase (20, 22-24). The low $K_m$ suggests that sinefungin or an analogue, if present in vivo, may regulate macrocin O-methyltransferase specifically in tylosin biosynthesis of S. fradiae (25).

**Kinetic Patterns and Reaction Mechanism**—Since the O-methyltransferase catalyzes stoichiometric conversion of macrocin to tylosin (Fig. 4) or of lactenocin to desmycosin (data not shown), no shunt metabolic route was operative for either conversion under the reaction conditions. Separability of tylosin and desmycosin from each other and from other macroclide compounds (as substrate, inhibitor, and/or internal standard) by the HPLC procedure (9) permits highly reproducible (i.e. ±2% between duplicate analyses) determination of the enzyme activity. Macrocin O-methyltransferase-catalyzed formation of tylosin (or desmycosin) from macrocin (or lactenocin) is linear with time within 10 min from reaction initiation and thus provides a reliable representation of initial activity (i.e initial velocity) in our kinetic studies.

The kinetic patterns that were used in determining a kinetic mechanism for macrocin O-methyltransferase-catalyzed methylation are summarized in Table VI. In the absence of an inhibitor, the intercepting substrate-interaction pattern for macrocin O-methyltransferase-catalyzed conversion of macrocin to tylosin indicates that the enzyme follows a sequential mechanism (26); i.e. both macrocin and AdoMet must bind to the O-methyltransferase prior to release of either tylosin or AdoHcy from the enzyme. The binding order of the two substrates can be derived from the inhibition patterns of demethylmacrocin and sinefungin (Table VI), which are presumed to be dead-end inhibitors of macrocin O-methyltransferase. The inhibition of the O-methyltransferase by demethylmacrocin and sinefungin is competitive with regard to macrocin and AdoMet, respectively. Thus, the uncompetitive inhibition by demethylmacrocin with respect to AdoMet and the noncompetitive inhibition by sinefungin with respect to macrocin become diagnostic of a binding order of the O-methyltransferase with AdoMet as the leading substrate (13).

Consistent with this binding order, the competitive inhibition with variable macrocin and the uncompetitive inhibition with variable AdoMet are also revealed by demethyllactenocin. The order of release of tylosin and AdoHcy from macrocin O-methyltransferase can be derived from the inhibition patterns of desmycosin and AdoHcy (Table VI). The inhibition of the O-methyltransferase by AdoHcy is competitive with regard to AdoMet and noncompetitive with regard to macrocin. Desmycosin was used as a macrolide product analogue in the inhibition study of macrocin O-methyltransferase-catalyzed conversion of macrocin → tylosin, so that the enzyme activity could be determined reproducibly by monitoring tylosin formation with HPLC. The inhibition of the O-methyltransferase by desmycosin is noncompetitive with respect to either macrocin or AdoMet. Since either desmycosin or tylosin is a weak macrolide inhibitor (Tables IV and VI) and either macrocin or lactenocin is an effective macrolide substrate (Table III), the same noncompetitive inhibition pattern of the O-methyltransferase by desmycosin may apply to both macrocin → tylosin and lactenocin → desmycosin conversions. That this analysis appears to be the case is consistent with the noncompetitive inhibition of the macrocin O-methyltransferase-catalyzed conversion of lactenocin → desmycosin by tylosin (also used as a macrolide product analogue) with regard to lactenocin. The product inhibition patterns not only substantiate the binding order of macrocin O-methyltransferase with AdoMet as the leading substrate but also support an order of release from the enzyme with AdoHcy as the last product. From all kinetic patterns as described above, we conclude that macrocin O-methyltransferase-catalyzed methylation of macrocin follows Ordered Bi Bi (Scheme 1) as the kinetic reaction mechanism. The intercepting substrate interaction pattern with lactenocin as the macrolide substrate (data not shown) and the noncompetitive inhibition of the lactenocin → desmycosin conversion by tylosin suggest that

![Scheme 1](image-url)
macrocin O-methyltransferase-catalyzed methylation of lactenocin follows an analogous Ordered Bi Bi reaction mechanism.

**Metabolic Inhibition of Macrocin O-Methyltransferase as Regulation of Tylosin Biosynthesis**—Macrocin O-methyltransferase is subject to metabolic inhibition by both competitive and noncompetitive patterns with respect to macrocin (Table VI). Thus, demethylmacrocin or demethyllactenocin (macrolide intermediate) may exhibit a moderate inhibitory effect at the active site of the enzyme. In contrast, tylosin or desmycosin (macrolide reaction product) or possibly relomycin (tylosin analogue and shunt metabolite) may exert a weak inhibitory effect at a regulatory site of the enzyme. The broad metabolic inhibition of macrocin O-methyltransferase could be a factor to the rate-limiting conversion of macrocin to tylosin and may play a regulatory role in tylosin biosynthesis of *S. fradiae*. Regardless of either inhibition mode, significance of the metabolic regulation might be overcome by increasing the O-methyltransferase activity via a "gene cloning" approach, which is being pursued (17, 18). The specific inhibition by tylosin, the major macrolide product, possibly at a regulatory site of macrocin O-methyltransferase provides a practical model for yield improvement of this industrially important antibiotic by site-directed modification of the enzyme.

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**EXPERIMENTAL PROCEDURES**

**Macrocin.** Highly purified macrocine compounds, streptomycin, and gentamicin were purchased from Sigma Chemical Company, St. Louis, MO. (except lactenocin, which was a gift of Drs. G. M. Wild and G. A. Stone, respectively). Ethanolamine, *S.* cridococcus (Lautman, 1965), and *S.* fradiae (Lautman, 1970) were obtained from the American Type Culture Collection, Rockville, MD.

**Experimental Procedures.** The purification of macrocin O-methyltransferase was performed as described. The purity of the enzyme was determined by native polyacrylamide gel electrophoresis and SDS-polyacrylamide gel electrophoresis, and the molecular weight of the enzyme was estimated to be 55,000 (1) by gel filtration chromatography. The enzyme was purified to homogeneity by a combination of ion exchange and size exclusion chromatography on a Sephadex G-25 column (2.5 X 100 cm) previously equilibrated with *PBE* buffer. Protein elution was monitored with the use of a Spectra-Physics 2000 UV absorbance detector.

**References.**
Macrocin O-Methyltransferase

RESULTS

Enzyme Stability. Macrocin O-methyltransferase from crude extracts of *S. globisporus*, as prepared at 4°C in 100 mM MOPS, pH 5.5, was stable with a half-life of two hours. Addition of 0.2 mM AdoMet, 2 mM PAPS, and 10% ethanol to the extract kept the O-methyltransferase stable for one week, after which reactivation of the enzyme occurred with a half-life of about 10 days. The O-methyltransferase became more stable after each chromatographic step of the enzyme purification (data not shown). The highly-purified enzyme (Table 1, step 4) was stable over six months at 4°C and pH 7.0 in the presence of 0.5 mM EDTA and 10% ethanol. The enzyme was stable at 4°C and pH 8.0-9.0 for about three days, and at 31°C and pH 7.5 (i.e., the reaction conditions) for only 11 min.

Enzyme assays. The four-step chromatographic procedure for macrocin O-methyltransferase purification is summarized in Table 1. The activity and protein peaks from Mono Q FPLC were essentially separable (Fig. 2). When analyzed by SDS-PAGE, the Mono-Q eluate (Table 1, step 4) migrated as a single protein band (Fig. 3).

Table of Contents. The molecular weight of macrocin O-methyltransferase as determined by gel filtration with a Sephadex G-200 column (1.5×100 cm), was 63,000 (Fig. 3). SDS-PAGE of the enzyme revealed a single protein band corresponding to a molecular weight of 32,000 (Fig. 2). The isoelectric point of the enzyme was estimated as 4.5 (data not shown).

Amino Acid Analysis. The amino acid composition of macrocin O-methyltransferase, as shown in Table II, was essentially indistinguishable from that derived from the nucleotide sequence of its structural gene (C. L. Hershberger, personal communication).

Other Methods. Procedures for SDS-PAGE, electrospray mass analysis, and amino acid analysis are those as described previously (15).
Macrolin O-Methyltransferase

substrate (Fig 6A). The same inhibition patterns were also observed with demethylactenocin (data not shown). In contrast, the inhibition of macrocin O-methyltransferase by simfungin, also used as a dead-end inhibitor, was competitive with AdoMet as the variable substrate (Fig 7A) and noncompetitive with macrocin as the variable substrate (Fig 7B). All secondary slope and intercept replots displayed linear inhibition. The $K_v$ and $K_i$ values, as calculated from the three kinetic inhibition programs, are shown in Table VI of the standard pent section. The two kinetic constants for demethylmacrolide were very similar to those for demethylactenocin. The $K_v$ value of either competitive macroinhibitor (demethylmacrolide or demethylactenocin) was close to the $K_v$ value of the macrolide substrate (macroc) in AdoMet, whereas macrocin concentration was fixed at 30 pM (B). In contrast to macrocin and lactenocin, the $K_v$ values of either macrocin or demethylactenocin was 10-fold higher than those of demethylactenocin (Fig 5B) and demethylmacrolide (Fig 5A) as the variable substrate (Fig 5B). All secondary slope and intercept replots displayed linear inhibition. The inhibition constants of either macrocin or lactenocin was 10-fold higher than those of demethylactenocin (Table VI of the standard pent section). With respect to a macrolide substrate, the $K_v$ value of AdoMet was 10-fold higher than that of demethylactenocin or demethylmacrolide. Also, the $K_v$ values of AdoMet and demethylactenocin were 3-16-fold higher than those of demethylactenocin. With regard to AdoMet, the $K_v$ of AdoMet was 8-16-fold higher than that of macrocin. However, the $K_v$ of AdoMet was indistinguishable from the $K_v$ of macrocin.