ELSEVIER

Biophysical Chemistry 74 (1998) 107–115

Biophysical Chemistry

Calculation of concentrations of equilibrium components in an in vitro activity test of vancomycin antibiotics and the possible mode of action

Husheng Yan*, Xiaohui Cheng, Binglin He

Institute of Polymer Chemistry, The State Key Laboratory of Functional Polymer Materials for Adsorption and Separation, Nankai University, Tianjin 300071, People's Republic of China

Received 9 April 1998; received in revised form 19 May 1998; accepted 19 May 1998

Abstract

The vancomycin group of antibiotics is considered to act by binding the bacterial cell wall mucopeptide precursor terminating in -L-Lys–D-Ala–D-Ala. The dimerization of these antibiotics is also believed to play a role in the action. In this paper, we analyzed the equilibria in the in vitro antibacterial activity test of the vancomycin antibiotics both with and without the cell wall precursor analogue di-acetyl-L-Lys–D-Ala–D-Ala (DALAA). Based on the equilibria and concentration balance, we obtained 10 equations (seven quadratic equations and three linear equations) containing 10 equilibrium concentrations which relate to the antibiotic, cell wall precursor and DALAA. A computer program was written to solve these equations from known dimerization constant and the binding constants (both monomer and dimer) with DALAA of the antibiotic. The concentrations in the test for vancomycin and eremomycin were obtained. The antibiotic activity of these antibiotics may be quantitatively correlated with their dimerization constants and the binding constants through the calculation. By analyzing the calculated results, we concluded that the cell wall-bound dimer may be the major contributor to the antibiotic activity in the case of eremomycin, while the cell wall-bound monomer is possibly the determinant for the activity of vancomycin.

Keywords: Vancomycin; Eremomycin; Antibiotic; Glycopeptide; Computer program

1. Introduction

The vancomycin group of antibiotics is clinically important for the treatment of Gram-positive infections, especially those caused by methicillin resistant *Staphylococcus aureus* [1–3]. The mechanism of antibiotic activity of the antibiotics is of great interest, both for the clinical importance mentioned above and because of their complexes with small peptides providing an excellent system for studying substrate–receptor interactions [4–6]. Nieto and Perkins [7] found that mucopeptide precursor molecules of the bacterial

* Corresponding author.

0301-4622/98/$19.00
PII S0301-4622(98)00172-0

cell wall containing the terminal -L-Lys–D-Ala–D-Ala fragment bound strongly to vancomycin. The attachment of the bulky vancomycin molecules to the precursor molecules terminating in L-Lys–D-Ala–D-Ala would prevent their incorporation into the cell wall structure, and the ability of vancomycin to form complexes with these peptides which are unique to the bacteria was linked to the mechanism of action of the antibiotic. The molecular basis of the binding of cell wall analogues to a vancomycin group of antibiotics was extensively investigated using small peptides (2–5 residues) [8–10]. Although the affinity of these antibiotics for bacterial cell wall analogues does correlate with in vitro antibiotic activity in some cases [9], it has been shown that this is not always the case [11]. For instance, it has been shown that eremomycin is consistently more active than vancomycin against a range of staphylococcal strains, despite the former having a lower affinity for the cell wall analogue di-acetyl-L-Lys–D-Ala–D-Ala (DALAA). Furthermore, as DALAA is a bacterial cell wall mimic, the addition of DALAA to the in vitro tests of the glycopeptide activity should reduce the activity of the antibiotics because of the competition between DALAA and normally synthesized cell wall components for the antibiotic binding site. Indeed, the addition of DALAA to the in vitro antibiotic activity tests of vancomycin decreased the activity. However, the addition of DALAA unexpectedly increased the activities of eremomycin in a low concentration of DALAA [11,12].

NMR experiments showed that most of the vancomycin group of antibiotics and their complexes with DALAA examined formed hydrogen bounded dimers, and there existed a positive cooperativity between the glycopeptide dimerization and the ligand binding [13–16]. Those which dimerize strongly showed anomalously high in vitro activity. Therefore the dimerization was believed to play a role in the mode of action of these antibiotics [12,14–16]. Furthermore, a model, the dimer binds to adjacent cell wall peptides of bacteria, for the action of the antibiotics was proposed [12,16]. However, the mechanism of the antibiotic activity is far from clear.

If concentrations of the various species in the in vitro test of the glycopeptide activity could be obtained, they would be useful for the elucidation of the mechanism of the antibiotic activity. However, the concentrations of some of the species are difficult to determine directly. And no attempt has been made to analyze or calculate the equilibrium concentrations in the test based on the chemical equilibria. In this paper, we thoroughly analyzed equilibria in the in vitro test of the glycopeptide activity in the presence or the absence of DALAA. This is a multi-components and multi-equilibria system. Based on the equilibria and concentration balance, 10 equations containing 10 equilibrium concentrations in the presence of DALAA were obtained. These equations are difficult to solve directly. Therefore a computer program was written to solve these equations. Based on the calculated data, the possible mode of the action for vancomycin and eremomycin was proposed.

2. Method

In the in vitro antibiotic activity test of the vancomycin group of antibiotics, all equilibrium components related to the antibiotic and the bacterial cell wall mucopeptide precursor are included in equilibria [Eqs. (1a)–(4a)]. The association constants corresponding to equilibria [Eqs. (1a)–(3a)] are expressed using Eqs. (1b)–(3b). There is no simple expression for equilibrium [Eq. (4a)] like the others and so, in the following quantitative analysis, equilibrium [Eq. (4a)] and ~LAAL~ are not considered (see Section 3).

$$2A \rightleftharpoons AA \tag{1a}$$

$$K_{dim} = \frac{[AA]}{[A]^2} \tag{1b}$$

$$\sim L + A \rightleftharpoons \sim LA \tag{2a}$$

$$K_2 = \frac{[\sim LA]}{[\sim L][A]} \tag{2b}$$

$$\sim L + AA \rightleftharpoons \sim LAA \tag{3a}$$

$$K_3 = \frac{[\sim LAA]}{[\sim L][AA]} \tag{3b}$$

$$\sim LAA + \sim L \rightleftharpoons \sim LAAL \sim \tag{4a}$$

where A represents the antibiotic, ~ L represents the cell wall mucopeptide precursor (ligand). When DALAA is added in the test, some other components will form and are included in equilibria [Eqs. (5a)–(8a)], and the corresponding association constants are expressed using Eqs. (5b)–(8b).

$$L + A \rightleftharpoons LA \tag{5a}$$

$$K_5 = \frac{[LA]}{[L][A]} \tag{5b}$$

$$L + AA \rightleftharpoons LAA \tag{6a}$$

$$K_6 = \frac{[LAA]}{[L][AA]} \tag{6b}$$

$$L + LAA \rightleftharpoons LAAL \tag{7a}$$

$$K_7 = \frac{[LAAL]}{[L][LAA]} \tag{7b}$$

$$\sim L + LAA \rightleftharpoons \sim LAAL \tag{8a}$$

$$K_8 = \frac{[\sim LAAL]}{[\sim L][LAA]} \tag{8b}$$

where L (ligand) represents DALAA. For the concentration balance of the solution, the following equations are obtained:

$$[A]_T = [A] + [\sim LA] + [LA] + 2([AA] + [\sim LAA] + [LAA] + [LAAL] + [\sim LAAL]) \tag{9}$$

$$[\sim L]_T = [\sim L] + [\sim LA] + [\sim LAA] + [\sim LAAL] \tag{10}$$

$$[L]_T = [L] + [LA] + [LAA] + 2[LAAL] + [\sim LAAL] \tag{11}$$

where $[A]_T$ is the total concentration of the antibiotic, $[\sim L]_T$ is the total concentration of the precursor on the cell wall, $[L]_T$ is the total concentration of L (DALAA). We assume that the cell wall mucopeptide precursor has the same affinity for the antibiotic (both monomer and dimer, respectively) as the precursor analogue L (DALAA). Mackay et al. [16] reported that the constants of the binding of the first and the second ligand to the dimer are equal or very similar. Therefore the following assumptions are made:

$$K_2 = K_5 = K_a$$

$$K_3 = K_6 = K_7 = K_8 = K_a'$$

where K_a is the binding constant of the monomer with L (DALAA), K_a' is the binding constant of the dimer with L (DALAA).

In the test, the values of $[A]_T$ and $[L]_T$ should be known. We assume that the value of $[\sim L]_T$ (related with bacteria in the test) is known. The values of K_a, K_a', and K_{dim} for vancomycin and eremomycin have been determined [16]. From these known values, Eqs. (1b)–(3b),(5b)–(8b),(9)–(11) are difficult to solve directly. However, if values of [A] and [L] are given, we can obtain [AA] using Eq. (1b), [LA] using Eq. (5b), and thus [LAA] using Eq. (6b) and [LAAL] using Eq. (7b). By combining Eqs. (2b),(3b),(8b),(10), and rearranging, we obtain:

$$[\sim L] = [\sim L]_T/(1 + K_a[A] + K_a'[AA] + K_a'[LAA])$$

so the value of $[\sim L]$ can be obtained. Then the values of $[\sim LA]$, $[\sim LAA]$ and $[\sim LAAL]$ can be calculated using Eqs. (2b),(3b),(8b), respectively. The values of $[A]_T$ and $[L]_T$ (which may be different from the initial known values of $[A]_T$ and $[L]_T$, respectively) can be obtained using Eqs. (9),(11), respectively. Based on this principle, we have written a computer program (by Qbasic language in Dos 6.22) by which all the equilibrium concentrations can be calculated using Eqs. (1b)–(3b),(5b)–(8b),(9)–(11) and the known values of K_a, K_a', K_{dim}, $[A]_T$, $[\sim L]_T$ and $[L]_T$. The procedure is described as follows: a larger value of [A], denoted as $[A]_1$, e.g. $[A]_1 = 1000[A]_T$, and a smaller value of [A], denoted as $[A]_2$, e.g. $[A]_2 = 0.001[A]_T$, are given. From the values of $[A]_1$ and $[A]_2$, the corresponding values of $[A]_T$, denoted as $[A]_{T1}$ and $[A]_{T2}$, can be calculated, respectively if a value of [L], denoted as $[L]_3$ (see below), is also given based on above method. The values of $[A]_1$ and $[A]_2$ are chosen in such a way so that the initial known value of $[A]_T$ must be between the values of $[A]_{T1}$ and $[A]_{T2}$. Then let $[A]_3 = ([A]_1 + [A]_2)/2$, and the corresponding value of $[A]_T$, denoted as $[A]_{T3}$, can be obtained in the same way. When the value of $[A]_{T3}$ is less than the initial known value of $[A]_T$, the value of $[A]_2$ is

omitted and the value of $[A]_3$ is taken as a new value of $[A]_2$; or when the value of $[A]_{T3}$ is larger than the initial known value of $[A]_T$, the value of $[A]_1$ is omitted and the value of $[A]_3$ is taken as a new value of $[A]_1$, and then above steps are repeated until the value of $[A]_{T3}$ is equal to the initial known value of $[A]_T$. The concentrations obtained in the last repeat are kept. Similarly, a larger value of [L], denoted as $[L]_1$, e.g. $[L]_1 = 10[L]_T$, and a smaller value of [L], denoted as $[L]_2$, e.g. $[L]_2 = 0.1[L]_T$, are given, and let $[L]_3 = ([L]_1 + [L]_2)/2$. This value of $[L]_3$ is used for the calculation as mentioned above. Then the corresponding value of $[L]_T$, denoted as $[L]_{T3}$, can be obtained based on Eq. (11). When the value of $[L]_{T3}$ is less than the initial known value of $[L]_T$, the value of $[L]_2$ is omitted and the value of $[L]_3$ is taken as a new value of $[L]_2$; or when the value of $[L]_{T3}$ is larger than the initial known value of $[L]_T$, the value of $[L]_1$ is omitted and the value of $[L]_3$ is taken as a new value of $[L]_1$. Then a new value of $[L]_3$ can be obtained from the new values of $[L]_1$ and $[L]_2$, and above steps are repeated until the value of $[L]_{T3}$ is equal to the initial known value of $[L]_T$. In this way, when both $[A]_{T3}$ and $[L]_{T3}$ are equal to the initial known values of $[A]_T$ and $[L]_T$, respectively, the corresponding concentrations, [A], [L], [AA], [LA], [LAA], [LAAL], [~L], [~LA], [~LAA] and [~LAAL], are the solution of Eqs. (1b)–(3b),(5b)–(8b),(9)–(11) from the known values of K_a, K_a', K_{dim}, $[A]_T$, $[\sim L]_T$ and $[L]_T$.

Similarly, computer programs have been written by which the total antibiotic concentrations, $[A]_T$, can be calculated at constant values of ([~LAA] + [~LAAL]) or ([~LA]/60 + [~LAA] + [~LAAL]), respectively.

3. Results and discussion

3.1. Analysis of the equilibria in the antibiotic test

In the in vitro antibiotic activity test of the vancomycin antibiotics, all the components related with the antibiotic and the cell wall precursor are included in equilibria [Eqs. (1a)–(4a)] when L (DALAA) is absent. When L (DALAA) is added in the test, new components LA, LAA, LAAL and ~LAAL would form, and thus the concentrations of the antibiotic monomer, A, and free dimer, AA, should decrease comparing with the case without L (DALAA) (due to the equilibrium movements, cf. the equilibria above). Subsequently, the concentrations of ~LA, ~LAA and ~LAAL~, which may contribute to the activity, would decrease based on the equilibria. However, it has been shown that low concentration of L (DALAA) increased the antibiotic activity for eremomycin [11,12]. Among the newly formed components, LA, LAA, LAAL and ~LAAL, only ~LAAL may contribute to the activity. Therefore the formation of ~LAAL may be the reason for the activity increasing with the addition of L (DALAA) in the case of eremomycin. As mentioned in Section 1, the dimerization was believed to play a role in the mode of action of these antibiotics. Therefore we postulate that all the dimers bound to the bacterial cell wall, including ~LAA, ~LAAL and ~LAAL~, may contribute to the antibiotic activity in the case of eremomycin.

The equilibria [Eqs. (1a)–(3a),(5a)–(8a)] should definitely exist in the test. However, ~LAAL~ may form {equilibrium [Eq. (4a)]} only when the cell wall precursors are locally concentrated. Where ~LAAL~ exists, it should be much more stable than the other two cell wall bound dimers if the two adjacent cell wall ligands are located in exactly proper positions for chelate forming, because the binding of the second equivalent of the antibiotic to the cell wall would be effectively intramolecular (intramolecular reactions can be accelerated by up to 10^9 relative to their intermolecular counterparts) [16,17]. It seems that the ratio of such pairs of cell wall precursors to the total ones may be small for eremomycin, otherwise the concentration of ~LAAL~ should be significantly higher relative to the other cell wall-bound dimers (as the former is much more stable than the later) and therefore the concentrations of the latter would be negligible relative to that of the former, and thus the addition of L (DALAA) would have not increased the activity. As the concentration of such pairs is unknown and, as

shown below, ~LAAL~ is probably not the major contributor to the antibiotic activity, equilibrium [Eq. (4a)] and ~LAAL~ are not considered in the quantitative analysis in this paper.

Table 1
Equilibrium concentrations (M) calculated[a]

	$[L]_T$	[L]	[A]	[AA]	[LA]	[LAA]
Vancomycin	0	0	9.40×10^{-7}	6.19×10^{-10}	0	0
	1×10^{-6}	5.62×10^{-7}	5.18×10^{-7}	1.88×10^{-10}	4.37×10^{-7}	2.22×10^{-10}
	2×10^{-6}	1.35×10^{-6}	3.19×10^{-7}	7.12×10^{-11}	6.47×10^{-7}	2.02×10^{-10}
	3×10^{-6}	2.25×10^{-6}	2.22×10^{-7}	3.47×10^{-11}	7.50×10^{-7}	1.64×10^{-10}
	5×10^{-6}	4.15×10^{-6}	1.36×10^{-7}	1.29×10^{-11}	8.45×10^{-7}	1.12×10^{-10}
	1×10^{-5}	9.08×10^{-6}	6.76×10^{-8}	3.20×10^{-12}	9.21×10^{-7}	6.10×10^{-11}
	2×10^{-5}	1.90×10^{-5}	3.36×10^{-8}	7.89×10^{-13}	9.59×10^{-7}	3.16×10^{-11}
	5×10^{-5}	4.90×10^{-5}	1.34×10^{-8}	1.25×10^{-13}	9.82×10^{-7}	1.29×10^{-11}
	1×10^{-4}	9.90×10^{-5}	6.66×10^{-9}	3.11×10^{-14}	9.90×10^{-7}	6.46×10^{-12}
Eremomycin	0	0	3.33×10^{-7}	3.32×10^{-7}	0	0
	1×10^{-6}	9.95×10^{-7}	3.27×10^{-7}	3.22×10^{-7}	1.26×10^{-9}	1.24×10^{-8}
	2×10^{-6}	1.97×10^{-6}	3.22×10^{-7}	3.11×10^{-7}	2.47×10^{-9}	2.39×10^{-8}
	3×10^{-6}	2.95×10^{-6}	3.16×10^{-7}	3.00×10^{-7}	3.64×10^{-9}	3.46×10^{-8}
	4×10^{-6}	3.94×10^{-6}	3.11×10^{-7}	2.90×10^{-7}	4.77×10^{-9}	4.45×10^{-8}
	5×10^{-6}	4.92×10^{-6}	3.05×10^{-7}	2.79×10^{-7}	5.85×10^{-9}	5.36×10^{-8}
	1×10^{-5}	9.83×10^{-6}	2.78×10^{-7}	2.32×10^{-7}	1.07×10^{-8}	8.88×10^{-8}
	2×10^{-5}	1.97×10^{-5}	2.30×10^{-7}	1.59×10^{-7}	1.77×10^{-8}	1.22×10^{-7}
	5×10^{-5}	4.94×10^{-5}	1.44×10^{-7}	6.23×10^{-8}	2.78×10^{-8}	1.20×10^{-7}
	1×10^{-4}	9.92×10^{-5}	8.60×10^{-8}	2.22×10^{-8}	3.33×10^{-8}	8.58×10^{-8}
	2×10^{-4}	1.99×10^{-4}	4.70×10^{-8}	6.63×10^{-9}	3.65×10^{-8}	5.15×10^{-8}
	5×10^{-4}	4.99×10^{-4}	1.98×10^{-8}	1.18×10^{-9}	3.86×10^{-8}	2.29×10^{-8}

	[LAAL]	[~L]	[~LA]	[~LAA]	[~LAAL]
Vancomycin	0	4.15×10^{-8}	5.85×10^{-8}	5.39×10^{-11}	0
	2.62×10^{-10}	5.62×10^{-8}	4.37×10^{-8}	2.22×10^{-11}	2.62×10^{-11}
	5.74×10^{-10}	6.76×10^{-8}	3.24×10^{-8}	1.01×10^{-11}	2.87×10^{-11}
	7.73×10^{-10}	7.49×10^{-8}	2.50×10^{-8}	5.46×10^{-12}	2.58×10^{-11}
	9.80×10^{-10}	8.31×10^{-8}	1.69×10^{-8}	2.25×10^{-12}	1.96×10^{-11}
	1.16×10^{-9}	9.08×10^{-8}	9.21×10^{-9}	6.10×10^{-13}	1.16×10^{-11}
	1.26×10^{-9}	9.52×10^{-8}	4.80×10^{-9}	1.58×10^{-13}	6.31×10^{-12}
	1.32×10^{-9}	9.80×10^{-8}	1.96×10^{-9}	2.57×10^{-14}	2.65×10^{-12}
	1.34×10^{-9}	9.90×10^{-8}	9.90×10^{-10}	6.46×10^{-15}	1.34×10^{-12}
Eremonycin	0	9.86×10^{-8}	1.28×10^{-10}	1.278×10^{-9}	0
	4.75×10^{-10}	9.86×10^{-8}	1.26×10^{-10}	1.236×10^{-9}	4.751×10^{-11}
	1.83×10^{-9}	9.86×10^{-8}	1.24×10^{-10}	1.195×10^{-9}	9.180×10^{-11}
	3.98×10^{-9}	9.86×10^{-8}	1.22×10^{-10}	1.154×10^{-9}	1.329×10^{-10}
	6.83×10^{-9}	9.86×10^{-8}	1.19×10^{-10}	1.114×10^{-9}	1.710×10^{-10}
	1.03×10^{-8}	9.86×10^{-8}	1.17×10^{-10}	1.074×10^{-9}	2.061×10^{-10}
	3.41×10^{-8}	9.87×10^{-8}	1.07×10^{-10}	8.911×10^{-10}	3.417×10^{-10}
	9.37×10^{-8}	9.88×10^{-8}	8.88×10^{-11}	6.133×10^{-10}	4.706×10^{-10}
	2.31×10^{-7}	9.92×10^{-8}	5.58×10^{-11}	2.411×10^{-10}	4.643×10^{-10}
	3.32×10^{-7}	9.95×10^{-8}	3.34×10^{-11}	8.608×10^{-11}	3.331×10^{-10}
	4.00×10^{-7}	9.98×10^{-8}	1.83×10^{-11}	2.580×10^{-11}	2.003×10^{-10}
	4.47×10^{-7}	9.99×10^{-8}	7.72×10^{-12}	4.593×10^{-12}	8.939×10^{-11}

[a] $K_a = 1.5\times10^6\ M^{-1}$, $K_a' = 2.1\times10^6\ M^{-1}$, $K_{dim} = 700\ M^{-1}$ for vancomycin; $K_a = 3.9\times10^3\ M^{-1}$, $K_a' = 3.9\times10^4\ M^{-1}$, $K_{dim} = 3\times10^6\ M^{-1}$ for eremomycin [16]; $[A]_T = 1\times10^{-6}$ M, $[\sim L]_T = 1\times10^{-7}$ M.

3.2. Calculation of the equilibrium concentrations in the in vitro test

In the in vitro antibiotic active test of vancomycin antibiotics, if equilibrium [Eq. (4a)] is not considered, the 10 equilibrium concentrations related to the antibiotic, the bacterial cell wall mucopeptide precursor and exogenous L (DALAA) are included in Eqs. (1b)–(3b),(5b)–(8b),(9)–(11). The values of $[A]_T$ and $[L]_T$ should be known and the value of K_a, K_a' and K_{dim} for eremomycin and vancomycin have been determined [16]. We assume $[\sim L]_T$ is known, e.g. $[\sim L]_T = 10^{-7}$ M. Then the concentrations included in these equations can be calculated from these equations and the known association constants by a computer program (see Section 2). Table 1 shows the calculated results for vancomycin and eremomycin.

3.3. Application of the calculated data and possible mode of the action

As mentioned above, as ~LAAL~ is not considered, the cell wall-bound dimers include ~LAA and ~LAAL. As shown in Table 1, in the case of eremomycin, the concentration of the total cell wall bound dimers ($[\sim LAA] + [\sim LAAL]$) is approx. 10 times higher than that of the cell wall-bound monomer ($[\sim LA]$). This supports the assumption that the cell wall bound dimers are the major contributors to the antibiotic activity for eremomycin. Table 1 shows that both $[\sim LA]$ and $[\sim LAA]$ decrease with the addition of L (DALAA), while $[\sim LAAL]$ increases and then decreases as the L (DALAA) concentration is further increased. Fig. 1 is a plot of the relative concentration of the total cell wall-bound dimers, $([\sim LAA] + [\sim LAAL])/[\sim LAA]_0$ (the subscript 0 represents the case without L (DALAA), the same below), against the total concentration of L (DALAA), $[L]_T$. Here ~LAA is assumed to have the same activity as ~LAAL in the same concentrations. It can be seen that addition of L (DALAA) indeed, although slightly, increases the concentration of the total cell wall-bound dimers in the case of eremomycin, while the concentration of the total cell wall-bound

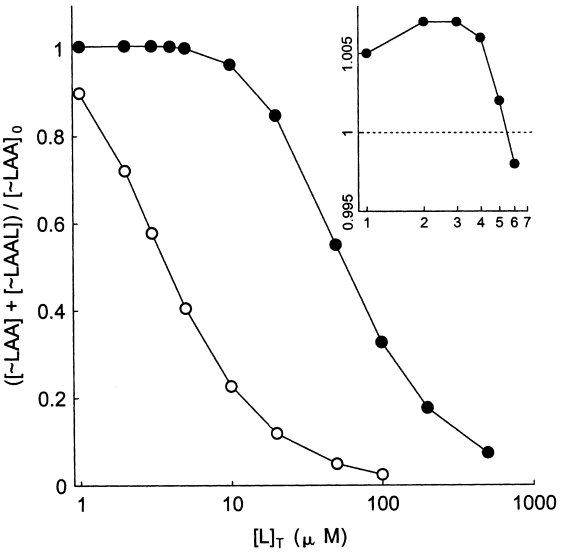

Fig. 1. Plot of relative concentration of the cell wall-bound dimers against DALAA concentration (data from Table 1). Inset: locally enlarged curve. ●, eremomycin; ○ vancomycin.

dimers of vancomycin decreased monotonically with the increasing L (DALAA) concentration, in good agreement with the previous findings [11,12]. If the total concentration of the cell wall precursor varies in a certain range, e.g. $[\sim L]_T = 1 \times 10^{-8}$ M or $[\sim L]_T = 1 \times 10^{-6}$ M, similar results were obtained (data not shown).

The calculation method should be more suitable for applying to the microdilution antibiotic activity test. Table 2 shows the calculated values of $[\sim LA]$, $[\sim LAA]$ and $[\sim LAAL]$ from the concentrations of added L (DALAA), $[L]_T$, and MICs (minimum inhibitory concentrations; in this paper, $[A]_T$ = MIC) of vancomycin and eremomycin against *Staphylococcus aureus* by microdilution test given in Good et al. [11]. It can be seen that, in the case of eremomycin, all the ratios of $([\sim LAA] + [\sim LAAL])$ to $[\sim LAA]_0$ remain unchanged (within twofold), in good agreement with observed results [11], supporting the hypothesis that the cell wall-bound dimers are the major contributor to the antibiotic activity for eremomycin. Fig. 2 shows the plot of the relative total concentration of antibiotic, $[A]_T/[A]_{T0}$ (corresponding relative MIC), against the total concentration of L (DALAA) when the values of

$([\sim LAA] + [\sim LAAL])/[\sim LAA]_0$ remain to be 1 (the concentrations of the total cell wall-bound dimers should be same at different concentrations of L (DALAA) at MICs if the bound dimers are the major contributor to the activity). The curve for eremomycin (Curve 1) is very close to the experimental plot of MIC against L (DALAA) concentration (Fig. 4 in Good et al. [11]). Therefore the total cell wall-bound dimers, $\sim$LAA and $\sim$LAAL, may indeed be the major contributor to the antibiotic activity for eremomycin. By exclusion, $\sim$LAAL$\sim$ possibly contributes little for the activity. If this was not the case, the great changes of the concentration of $\sim$LAA with the addition of L (DALAA), as shown in Table 2, would have caused the concentration of $\sim$LAAL$\sim$ to change much {cf. equilibrium [Eq. (4a)]} and thus the concentration of total cell wall bound dimer $([\sim LAA] + [\sim LAAL] + [\sim LAAL\sim])$ at MICs would have not remained unchanged (significantly exceeding twofold).

Table 2 shows that, in the case of vancomycin, however, the values of $([\sim LAA] + [\sim LAAL])/[\sim LAA]_0$ change markedly, and the concentration of $\sim$LAA is approx. 10 times lower than that for eremomycin in the absence of L (DALAA) at MICs (they should be similar if the cell wall-bound dimers were also the major contributor to the activity for vancomycin). Curve 2 in Fig. 2 deviates much from the experimental plot (Fig. 4 in Good et al. [11]). Table 1 also shows that eremomycin has higher $[\sim LAA]$ than vancomycin by a factor of 24 in the absence of L (DALAA), while the antibiotic activity of the former is only 2$\sim$5 times of that of the latter [11]. Subsequently, it seems that the $\sim$LAA is not the only contributor to the antibiotic activity for vancomycin in the absence of L (DALAA). As

Table 2
Comparison of MICs and calculated results

$[L]_T$ (μM)		0	8	16	32
Vancomycin	MIC ($[A]_T$) (μM)[a]	2	4	4	8
	$[\sim LA]$ (M)	7.74×10^{-8}	4.28×10^{-8}	2.34×10^{-8}	2.38×10^{-8}
	$[\sim LAA]$ (M)	1.40×10^{-10}	2.10×10^{-11}	4.66×10^{-12}	4.88×10^{-12}
	$[\sim LAAL]$ (M)	0	2.01×10^{-10}	1.20×10^{-10}	2.49×10^{-10}
	$([\sim LAA] + [\sim LAAL])/[\sim LAA]_0$	1.00	1.58	0.89	1.81
	$([\sim LA]/60 + [\sim LAA] + [\sim LAAL])/$ $([\sim LA]_0/60 + [\sim LAA]_0)$	1.00	0.65	0.36	0.45
Eremomycin	MIC ($[A]_T$) (μM)[a]	1	1	1	2
	$[\sim LA]$ (M)	1.28×10^{-10}	1.11×10^{-10}	9.55×10^{-11}	1.06×10^{-10}
	$[\sim LAA]$ (M)	1.28×10^{-9}	9.61×10^{-10}	7.10×10^{-10}	8.77×10^{-10}
	$[\sim LAAL]$ (M)	0	2.95×10^{-10}	4.36×10^{-10}	1.06×10^{-9}
	$([\sim LAA] + [\sim LAAL])/[\sim LAA]_0$	1.00	0.98	0.90	1.51
		64	128	256	512
Vancomycin	MIC ($[A]_T$) (μM)[a]	16	32	64	>64
	$[\sim LA]$ (M)	2.38×10^{-8}	2.32×10^{-8}	2.20×10^{-8}	
	$[\sim LAA]$ (M)	4.87×10^{-12}	4.62×10^{-12}	4.14×10^{-12}	
	$[\sim LAAL]$ (M)	4.93×10^{-10}	9.34×10^{-10}	1.67×10^{-9}	
	$([\sim LAA] + [\sim LAAL])/[\sim LAA]_0$	3.60	6.73	12.0	
	$([\sim LA]/60 + [\sim LAA] + [\sim LAAL])/$ $([\sim LA]_0/60 + [\sim LAA]_0)$	0.63	0.93	1.42	
Eremomycin	MIC ($[A]_T$) (μM)[a]	2	2	8	8
	$[\sim LA]$ (M)	6.83×10^{-11}	3.91×10^{-11}	4.27×10^{-11}	2.19×10^{-11}
	$[\sim LAA]$ (M)	3.64×10^{-10}	1.18×10^{-10}	1.43×10^{-10}	3.72×10^{-11}
	$[\sim LAAL]$ (M)	8.88×10^{-10}	5.84×10^{-10}	1.38×10^{-9}	7.31×10^{-10}
	$([\sim LAA] + [\sim LAAL])/[\sim LAA]_0$	0.98	0.55	1.19	0.60

[a] From Good et al. [11].

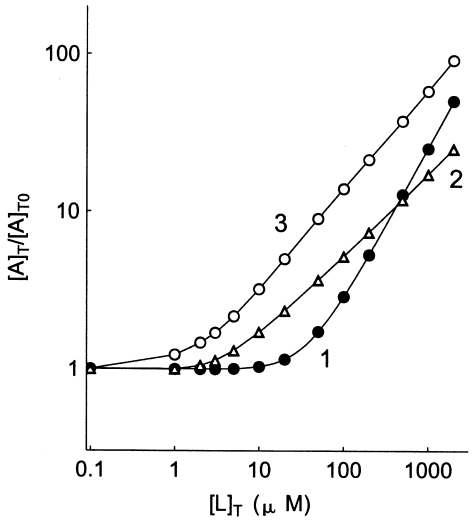

Fig. 2. Plot of the relative total concentration of the antibiotic against the DALAA concentration. ([~ LAA] + [~ LAAL])/[~ LAA]$_0$ = 1 for curve 1 (eremomycin) and curve 2 (vancomycin). ([~ LA]/60 + [~ LAA] + [~ LAAL])/([~ LA]$_0$/60 + [~ LAA]$_0$) = 1 for curve 3 (vancomycin). [~ LA]$_0$ and [~ LAA]$_0$ are obtained from Table 2.

mentioned above ~LAAL~ contributes little to the activity for eremomycin. The only reason for this may be that the concentration of the properly located adjacent cell wall precursor pairs are small. Therefore ~LAAL~ may also not be the major contributor to the activity for vancomycin. What is, then, the major contributor to the antibiotic activity for vancomycin? A possible candidate is the cell wall-bound monomer, ~LA. It can be seen from Tables 1 and 2 that the concentration of ~LA is higher than that of ~LAA by a factor of 500–1000 for vancomycin, while the former is approx. 10 times lower than the latter for eremomycin. The inhibition of cell wall synthesis by glycopeptides may be at least partially due to the steric hindrance of carbohydrate linking enzymes by the antibiotic which is bound to the peptide nearby [18]. Although the cell wall-bound monomer may possess smaller steric hindrance than the cell wall-bound dimer, the former is much more numerous than the latter in the case of vancomycin, and thus it is possible that the bound monomer is also contribute, in a lower magnitude, to the antibiotic activity. By comparing the data in Tables 1 and 2, we conclude that the bound dimer of eremomycin is approx. 60–90 times more active than the bound monomer of vancomycin. Then the value of ([~ LA]/60 + [~ LAA] + [~ LAAL]) should be the determinant for the antibiotic activity on the assumption that the bound dimer of eremomycin and that of vancomycin possess the same activities. Table 2 shows that the values of ([~ LA]/60 + [~ LAA] + [~ LAAL]) indeed do not change much. The corresponding curve (Curve 3 in Fig. 2) is closer to the experimental plot (Fig. 4 in Good et al. [11]).

In the analysis above we assume that the cell wall-bound dimers have the same activities for both eremomycin and vancomycin. However, the cell wall-bound dimer for different vancomycin antibiotics may be different, but the difference should be relative small compared to the difference between the cell wall-bound monomer and the cell wall-bound dimer. For example, eremomycin and MM47761 have similar dimerization and ligand binding affinities, however, the former is 2–5 times more active than the latter.

4. Conclusion

For the in vitro antibiotic activity test of vancomycin and eremomycin, 10 equilibrium concentrations related to the antibiotic, the bacterial cell wall precursor and exogenous L (DALAA) have been calculated by a computer program. The calculated data shown that the equilibrium concentration of the total cell wall-bound dimers, ~LAA and ~LAAL, increases to reach a plateau and then decreases with the increasing of L (DALAA) concentration for eremomycin, in good agreement with the observed result [11] that the antibacterial activity increased in the presence of low concentration of L (DALAA); the calculated relative concentrations of the total cell wall-bound dimers at MICs [11] remain unchanged. Together with the fact that the equilibrium concentration of the total cell wall-bound dimers is 10 times higher than that of the cell wall-bound monomer for eremomycin, we concluded that the cell wall-bound dimer may be the major contributor to the antibiotic activity of eremomycin. For van-

comycin, the calculated relative concentrations of the total cell wall-bound dimers at MICs [11] do not remain unchanged, together with the equilibrium concentration of the cell wall-bound monomer is higher than that of the total cell wall-bound dimers by a factor of 500–1000, indicating that the cell wall-bound monomer is possibly the major contributor to the activity of vancomycin.

References

[1] M.C. McHenry, T.L. Gavan, Pediatr. Clin. North Am. 30 (1983) 31.
[2] M. Foldes, R. Munro, T.C. Sorrell, S. Shankar, M.J. Toohey, J. Antibiot. Agents Chemother 11 (1990) 21.
[3] M.P. Wilhelm, Mayo. Clin. Proc. 66 (1991) 1165.
[4] D.H. Williams, J.P.L. Cox, A.J. Doig, et al., J. Am. Chem. Soc. 113 (1991) 7020.
[5] D.H. Williams, M.S. Searle, J.P. Mackay, U. Gerhard, R.A. Maplestone, Proc. Natl. Acad. Sci. USA 90 (1993) 1172.
[6] D.H. Williams, M.S. Searle, M.S. Westwell, U. Gerhard, S.E. Holroyd, Philos. Trans. R. Soc. London, Ser. A 354 (1993) 11.
[7] M. Nieto, H.R. Perkins, Biochem. J. 123 (1971) 789.
[8] G.M. Sheldrich, P.G. Jones, O. Kennard, D.H. Williams, G.A. Smith, Nature 271 (1978) 223.
[9] R. Kannan, C.M. Harris, T.M. Harris, J.P. Waltho, N.J. Skelton, D.H. Williams, J. Am. Chem. Soc. 110 (1988) 2946.
[10] D.H. Williams, M.P. Williamson, D.W. Butcher, S.J. Hammond, J. Am. Chem. Soc. 105 (1983) 1332.
[11] V.M. Good, M.N. Gwynn, D.C. Knowles, J. Antibiot. 43 (1990) 550.
[12] D.A. Beauregard, D.H. Williams, M.N. Gwynn, D.J. Knowles, Antimicro. Agents Chemother. 39 (1995) 781.
[13] J.P. Waltho, D.H. Williams, J. Am. Chem. Soc. 111 (1989) 2475.
[14] U. Gerhard, J.P. Mackay, R.A. Maplestone, D.H. Williams, J. Am. Chem. Soc. 115 (1993) 232.
[15] J.P. Mackay, U. Gerhard, D.A. Beauregard, R.A. Maplestone, D.H. Williams, J. Am. Chem. Soc. 116 (1994) 4573.
[16] J.P. Mackay, D.A. Beauregard, M.S. Westwell, M.S. Searle, D.H. Williams, J. Am. Chem. Soc. 116 (1994) 4581.
[17] M.L. Page, W.P. Jencks, Proc. Natl. Acad. Sci. USA 68 (1971) 1678.
[18] E.F. Gale, E. Cundliffe, P.E. Reynolds, M.H. Richard, The Molecular Basis of Antibiotic Action, Wiley, Bristol, 1981, p. 145.