COMPLEXATION OF ACETYL-D-ALANYL-
D-ALANINE BY ANTIBIOTIC A35512B

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Antibiotic A35512B is a member of the glycopeptide family of antibiotics; complete structures have been reported for two members of this group, vancomycin and ristocetin. Both vancomycin and ristocetin have been shown to form complexes with mucopeptides containing the terminal dipeptide D-alanyl-D-alanine; this interaction within the bacterial cell inhibits cross-linking of the cell wall and leads to eventual lysis of the organism. Chemical attack on A35512B produces degradation products which are closely related to those obtained from ristocetin, suggesting structural similarities between the two antibiotics. Thus, A35512B might also be expected to form complexes with mucopeptide analogs. This paper reports the observation of such a complex with acetyl-D-alanyl-D-alanine (Ac-D-Ala-D-Ala).

The interactions of glycopeptide antibiotics with acetyl-D-alanyl-D-alanine and related molecules have been examined by NMR methods (both vancomycin and ristocetin) and by UV difference spectroscopy (chiefly vancomycin). The UV difference method was used to examine complex formation between Ac-D-Ala-D-Ala and A35512B in the work reported here. In order to compare the binding results for A35512B with those for a more thoroughly characterized glycopeptide, an identical experiment was conducted using vancomycin.

A tandem-cell arrangement was used for titration of Ac-D-Ala-D-Ala into a solution of the antibiotic (A35512B or vancomycin). Initial concentrations were 2.00 $\times$ 10$^{-4}$ M Ac-D-Ala-D-Ala (0.404 mg/ml), 1.60 $\times$ 10$^{-4}$ M A35512B (0.313 mg/ml), and 1.72 $\times$ 10$^{-4}$ M vancomycin hydrochloride (0.256 mg/ml). The solvent for all solutions was 0.02 M sodium citrate buffer, pH 5.1. The total lightpath of each cell was 2 cm, and the initial volume of antibiotic solution was 0.8 ml in each case. Examples of the UV difference spectra produced by the addition of Ac-D-Ala-D-Ala to A35512B or to vancomycin are shown in Fig. 1.
For both antibiotics both positive and negative difference peaks are produced. The experimental property used in the calculation of equilibrium constants for peptide-glycopeptide complexation is the difference, \( \Delta \), between these positive and negative peaks. In Fig. 1, for example, \( \Delta = (A_{288} + B - A_{288}) = 0.0988 \) for \( A35512B \), and \( \Delta = (A_{294} + B - A_{281}) = 0.1138 \) for vancomycin. The observed values of \( \Delta \) are plotted as a function of the molar ratios of Ac-D-Ala-D-Ala to glycopeptide in Figs. 2 and 3.

The equilibrium constant for the complexation reaction \( A + P \rightleftharpoons C \) is given by

\[
K = \frac{C}{(A)(P)} = \frac{C}{(A'_0 - C)(P'_0 - C)}
\]

where \( C \) = concentration of the complex, \( A'_0 \) = total concentration of free and complexed antibiotic, and \( P'_0 \) = total concentration of free and complexed peptide.

(If the initial concentrations of antibiotic and peptide are \( A_0 \) and \( P_0 \) and the initial antibiotic volume is \( V_0 \) ml, then after the addition of \( V \) ml of peptide solution \( A'_0 = A_0(V_0 + V) \) and \( P'_0 = P_0(V)/(V_0 + V) \).)

The equilibrium constant expression can be solved for \( C \) to give expression (1):

\[
C = \frac{[A_0' + P_0' + (1/K)] - \sqrt{[A_0' + P_0' + (1/K)]^2 - 4(A_0')(P_0')(1/K)}}{2}
\]

If the value of \( \Delta \) which would be observed for a totally-complexed antibiotic at concentration \( A_0 \) is defined as \( D \), then at any point in the titration the fraction of antibiotic complexed is given by:

\[
\frac{D(V_0 + V)/V_0}{D} = \frac{C}{A_0'}
\]

which can be simplified to equation (2):

\[
\Delta = C(D/A_0).
\]

Substitution of (1) into (2) produces an expression for \( \Delta \) (the observed UV difference) as a function of \( V \) (the volume of peptide added), with \( D \) and \( K \) as adjustable parameters:

\[
\Delta = \frac{D}{2A_0} (A_0' + P_0' + (1/K)) - \sqrt{(A_0' + P_0' + (1/K))^2 - 4(A_0')(P_0')}
\]

(Note that both \( A_0' \) and \( P_0' \) are defined in terms of \( V \).)

The solid lines in Figs. 2 and 3 demonstrate the least-squares fit of equation (3) to the values of \( \Delta \) shown in the figures. For vancomycin, \( K = 1.4 \times 10^4 \text{ M}^{-1} \), which is identical with the result obtained from an NMR dilution study. For \( A35512B \), \( K = 7.3 \times 10^4 \text{ M}^{-1} \), indicating an even higher affinity for Ac-D-Ala-D-Ala than that exhibited by vancomycin. These data imply both a structural and a biological similarity between \( A35512B \) and other members of the glycopeptide antibiotics class. Structure elucidation studies on \( A35512B \) are in progress.

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References

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