A Study of the Binding of Vancomycin to Dipeptides using Capillary Electrophoresis

J. L. Carpenter,[a] P. Camilleri,*[b] D. Dhanak[b] and D. Goodall*[a]

[a] *Department of Chemistry, University of York, Heslington, York YO1 5DD, UK*
[b] *SmithKline Beecham, The Frythe, Welwyn, Herts AL6 9AR, UK*

Capillary electrophoresis (CE) has been used as a rapid technique for the determination of the absolute binding constants of dipeptides with vancomycin in solution; the values obtained for the mucopeptide mimic *N*-acetyl-D-Ala-D-Ala and related dipeptides are in good agreement with values reported in the literature using either differential UV spectrometry or NMR.

Vancomycin is a broad spectrum antibiotic first isolated from *Streptomyces orientalis*.[1] It is widely used clinically in the treatment of Gram-positive bacterial infections. It binds strongly[2,3] to mucopeptides terminating in the dipeptide-D-Ala-D-Ala. Several studies[4,5] have been carried out of the interaction of the cell wall mucopeptide mimic *N*-acetyl-D-Ala-D-Ala **1** and closely related peptides with vancomycin.

Binding assays have been developed using NMR[4] and differential UV spectrophotometry.[5] Smith *et al.*[6] describe an HPLC method whereby the binding of vancomycin to a number of dipeptide ligands can be studied simultaneously; this technique involves the preparation of a bromoacetyl resin and chemical linking of vancomycin to the resin before any studies can be carried out. This method allows only the determination of the relative extent of binding of dipeptides to the antibiotic and cannot be used to give accurate measurements of absolute binding constants.

The chemical structure[7] of vancomycin contains a secondary amine group, a primary amine on the sugar moiety and a carboxy group. These groups make this antibiotic charged over a wide pH range. Moreover a change in the net charge is expected to result on complexation with a dipeptide such as **1**.

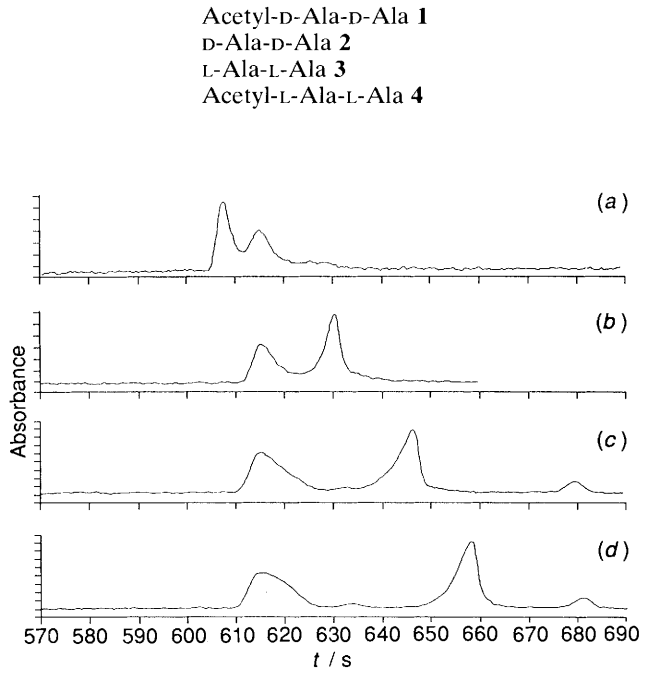

Fig. 1 Electropherograms of vancomycin (5.8×10^{-4} mol dm^{-3}), (*a*) in the absence and in the presence of D-Ala-D-Ala, (*b*) 3 mmol dm^{-3}, (*c*) 10 mmol dm^{-3} and (*d*) 20 mmol dm^{-3}. Buffer: 50 mmol dm^{-3} Tris HCl (pH 8.28). CE apparatus, Beckman P/ACE 2100; voltage, +10 kV; capillary, 57 cm (75 μm i.d.); absorbance, UV 260 nm; temperature, 298 K; injection, 1 s pressure.

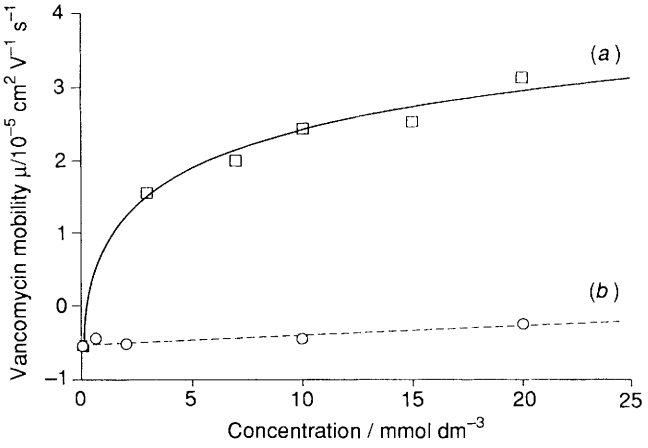

Fig. 2 Variation of vancomycin electrophoretic mobility in the presence of either (*a*) D-Ala-D-Ala, or (*b*) L-Ala-L-Ala

Table 1 A comparison of binding constants of vancomycin to dipeptides

Substrate	K/dm^3 mol^{-1}[a]	Technique	pH	Ref.
2	200	^{1}H NMR	10	4(*b*)
2	220 ± 60	CE	8.28	This study
3	~0	CE	8.28	This study
1	1×10^5	UV	—[b]	2
1	2.0×10^4	UV	5.1	5
1	$(3 \pm 1) \times 10^4$	UV	5.0	11
1	$(1.4 \pm 0.1) \times 10^4$	^{1}H NMR	5.5[c]	4(*a*)
1	$(1.1 \pm 0.5) \times 10^4$	CE	8.28	This study
4	~0	CE	8.28	This study

[a] Temperature 25 °C unless otherwise stated. [b] pH not stated; ambient temperature. [c] pD; 35 °C.

These changes can be ideally followed using the relatively novel analytical technique of capillary electrophoresis (CE).[8,9] In this communication we report preliminary results that demonstrate the feasibility of using CE to determine the binding or complexation constants of dipeptide ligands with vancomycin in solution.

Fig. 1 shows the electrophoretic behaviour of vancomycin in the absence and the presence of varying amounts of the non-acetylated form of **1**, D-Ala-D-Ala **2**. The peak that has a constant mobility with increasing ligand concentration is due to the neutral marker mesityl oxide, which moves solely under the influence of the electroosmotic flow of the bulk electrolyte. From Fig. 1 it is clear that vancomycin has a net positive charge in the absence of ligand, since it migrates faster than mesityl oxide. On increasing concentration of **2** the mobility of vancomycin decreases due to increasing complexation. In the CE background electrolyte buffer (pH 8.28) the vancomycin complex with **2** is expected to have a slightly net negative charge as the pK_a values of the secondary amine group, the primary amine on the amino sugar vancosamine moiety and the free amino group on **2** are in the range 8 to 10; the pK_a values of the two carboxy groups (one on the antibiotic and one on the ligand) are in the range 3.5 to 4.5 and are fully ionised at the pH of this study.

The variation of the mobility of vancomycin with different amounts of **2** and the isomeric dipeptide L-Ala-L-Ala **3** is plotted in Fig. 2. The change in mobility with ligand concentration follows a hyperbolic curve only in the case of **2**. From these plots it is clear that vancomycin has a much bigger affinity for **2** than **3**. Similar data were obtained for **1** and the acetylated analogue acetyl-L-Ala-L-Ala **4**. As expected[4] the complexing ability of **1** is greater than that of **2**. Moreover, in agreement with the literature[6] **4** does not appear to complex with vancomycin. Under the conditions of our experiments eqn. (1) applies where μ is the measured mobility of vancomycin at a concentration of ligand [L], μ_{max} is the limiting value of the mobility at a high concentration of L and K is the binding constant. The equation is derived on the assumption that vancomycin and L form a 1 : 1 complex. A Scatchard plot of μ/[L] against μ using the data from Fig. 2 gives values of μ_{max} and K from the intercept on the x-axis and the slope of the fitted line, respectively. Table 1 compares the binding constants obtained from this study with previously reported values measured by spectroscopic methods. There is good agreement between results from the present CE technique and previous methods. The results in Table 1 also confirm[6] the inability of the dipeptides **3** and **4** to bind to vancomycin.

$$\mu/[L] = \mu_{max}K - \mu K \quad (1)$$

The binding assay reported in this communication could prove to be valuable in determining binding constants of other ligands under near physiological conditions. It has been reported recently that ester bond formation between hydroxybutyric acid and D-alanine is catalysed by Van A (a 38 kDa membrane-associated protein) and may ultimately lead to vancomycin resistance.[10] Ligands such as *N*-acetyl-D-Ala-D-2-hydroxybutyrate **5** bind much less strongly to vancomycin than **1**. The study of the complexing ability of vancomycin and related glycopeptides to amino acid containing ligands by CE may be useful not only as an alternative method of determining binding constants but also in providing information on the net charge characteristics of these complexes. This additional information could be important in the design of novel vancomycin-related glycopeptides that are able to bind strongly even with molecules such as **5**.

We thank Dr M. Gilpin (SmithKline Beecham) and Dr D. H. Williams (University of Cambridge) for helpful discussions and the SERC for a CASE award to J. L. C.

Received, 10th March 1992; Com. 2/01297I

References

1 M. H. McCormick, W. H. Stark, C. E. Pittenger, R. C. Pittenger and J. M. McGuire, *Antibiotics Annual, 1955–56*, Medicinal Encyclopaedia Inc., New York, 1956, p. 606.
2 H. R. Perkins, *Biochem. J.*, 1969, **111**, 195.
3 M. Nieto and H. R. Perkins, *Biochem. J.*, 1971, **123**, 773.
4 (*a*) J. P. Brown, J. Feeney and A. S. V. Burgen, *Mol. Pharmacol.*, 1975, **11**, 119; (*b*) J. P. Brown, L. Terenius, J. Feeney and A. S. V. Burgen, *Mol. Pharmacol.*, 1975, **11**, 126.
5 M. Nieto and H. R. Perkins, *Biochem. J.*, 1971, **123**, 789.
6 P. W. Smith, G. Chang and W. Clark Still, *J. Org. Chem.*, 1988, **53**, 1590.
7 J. C. J. Barna and D. H. Williams, *Ann. Rev. Microbiol.*, 1984, **38**, 339.
8 R. A. Wallingford and A. G. Ewing, *Adv. Chromatogr.*, 1989, **29**, 1.
9 W. G. Kuhr, *Anal. Chem.*, 1990, **62**, 403R.
10 T. D. H. Bugg, G. D. Wright, S. Dutka-Malen, M. Arthur, P. Courvalin and C. T. Walsh, *Biochemistry*, 1991, **30**, 10 408.
11 M. P. Williamson, D. H. Williams and S. J. Hammond, *Tetrahedron*, 1984, **40**, 569.