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**Note**

# Acid-base properties and proton-speciation of vancomycin

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## Summary

The protonation-deprotonation equilibria of the glycopolypeptide antibiotic vancomycin are characterized in terms of protonation macroconstants, isoelectric point and diagram of pH-dependent species distribution. Vancomycin contains two basic and four acidic groups. Of these, dissociation of the C-terminal carboxyl group takes place separately at low pH, whereas proton binding of the two amino sites and deprotonation of the three phenolic hydroxyl groups occur between pH 5 and 13, in a highly overlapping fashion. Our study indicates for an isoelectric point of 8.30 the vancomycin molecule and an average charge of +0.67, with the predominant existence of penta- and tetraprotonated species at physiological pH.

Vancomycin belongs to the glycopeptide class of antibiotics. Since its discovery in 1956, it has been used as a therapeutically important agent in cases of Gram-positive infections, especially in penicilline resistance or allergy (Griffith, 1981). Vancomycin is a polyfunctional, amphoteric compound (Fig. 1), official in the British Pharmacopoeia (1988), USP XXII, and the Extra Pharmacopoeia (29th Edn).

Several aspects of the analytical and physical chemistry of vancomycin have been studied, such as quantitative determination in biological samples (e.g., see Kirchmeier and Upton, 1978; Bruce and McClain, 1982), chromatographic behaviour (e.g., Thomas and Newland, 1987), and its separation from co-fermentation and degradation products (e.g., Florena-Wasserman et al., 1987; Inman, 1987). The exact structure of vancomycin has been known since the late seventies (Williams and Kalman, 1977; Pfeiffer, 1981).

However, no data have appeared concerning its acid-base properties, despite the fact that the molecular conformation and several biological processes (for example, membrane penetration, receptor binding) are greatly influenced by the state of protonation.

In the present paper, we report data on three parameters of its acid-base chemistry: the protonation macroconstants, the isoelectric point and the distribution of its differently protonated species as a function of pH. All constants were determined by potentiometric titrations at 25 ± 0.1°C and near 0.2 M ionic strength. 20 ml aliquots

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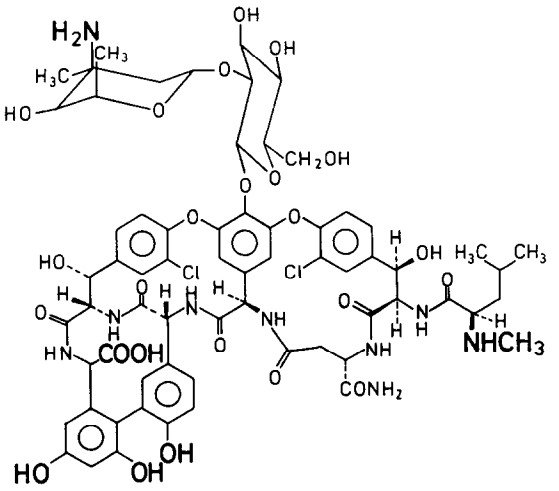

Fig. 1. The structure of vancomycin.

of 0.01 M vancomycin and 0.1 M HCl solutions were titrated by 1 M NaOH under an $N_2$ atmosphere. The electrode was calibrated with standard buffer solutions (Merck) in the range pH 2–12. Details of the experimental technique have been described earlier (Takács-Novák et al., 1990).

Vancomycin contains six functional groups (denoted in bold-face type; Fig. 1) which participate in acid-base equilibria. In strongly basic solution (pH > 13), the molecule bears four negative charges represented by three phenolate groups and one carboxylate. With decreasing pH, vancomycin can bind a total of six protons, altering the molecular charge to +2, by pH ≅ 0. The stepwise (successive) protonation macroconstants ($K_1, \ldots, K_6$) and the cumulative macroconstants ($\beta_1, \ldots, \beta_6$) are listed in Table 1 in log units. The estimated ambiguity of entries in Table 1 is based upon the standard deviation of three parallel potentiometric determinations and varies between 0.01 and 0.08 log $K$ units. The greatest uncertainty, belonging to log $K_1$ = log $\beta_1$ = 12.0,

TABLE 1

*Complex products and protonation macroconstants of vancomycin in log units at 25.0 ± 0.1°C and I = 0.2 ionic strength*

| Complex products | Stepwise macroconstants |
|---|---|
| log $\beta_1$ = 12.0 | log $K_1$ = 12.0 |
| log $\beta_2$ = 22.40 | log $K_2$ = 10.40 |
| log $\beta_3$ = 31.98 | log $K_3$ = 9.59 |
| log $\beta_4$ = 40.87 | log $K_4$ = 8.89 |
| log $\beta_5$ = 48.62 | log $K_5$ = 7.75 |
| log $\beta_6$ = 50.80 | log $K_6$ = 2.18 |

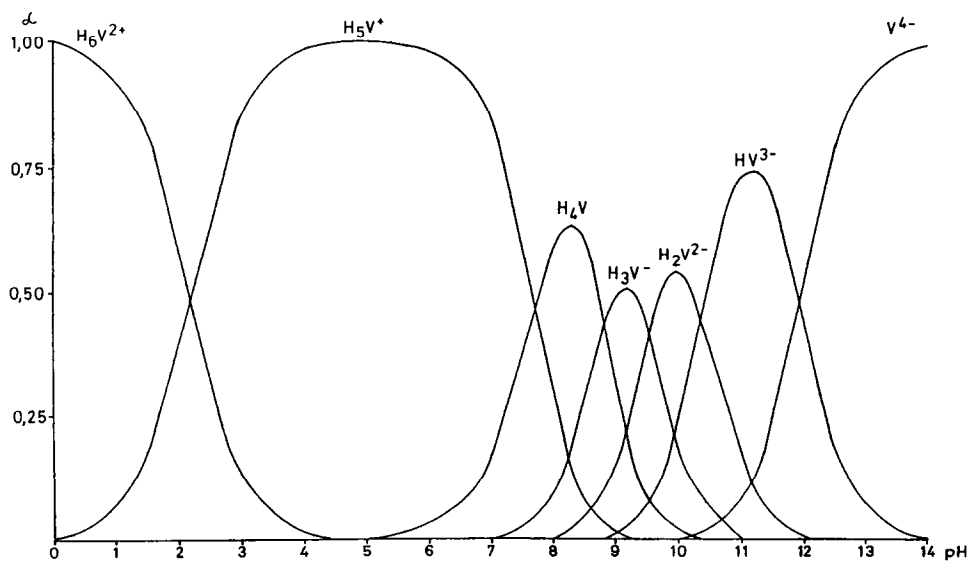


Fig. 2. Distribution of vancomycin macrospecies as a function of pH ($V^{4-}$ represents the totally deprotonated form; $H_4V$ refers to the neutral form and $H_6V^{2+}$ to the perprotonated species).

is due to the limited accuracy of pH measurement at very high pH.

Acid-base equilibrium processes can be observed in two different pH regions. Dissociation of the carboxyl group takes place at low pH and is well-separated from all the other protonation/deprotonation equilibria of the other five functional groups. Therefore, the value log $K_6 = 2.18$ can be assigned to the carboxylate proton-binding site with a high level of certainty. It should be borne in mind, however, that this value reflects the basicity only of the carboxylate when all other groups are in their acidic (ammonium or phenolic) form. Protonations of the phenolate and amino groups take place between pH 13 and 5 in multiply overlapping stages and are expressed in the stepwise macroconstants $K_1, \ldots, K_5$. Macroconstants, in principle, cannot be assigned to individual functional groups (Noszál, 1986, 1990; Noszál and Osztás, 1989), since the protonation of each group is represented more or less by every macroconstant. Nevertheless, from chemical considerations and analogies, it is clearly evident that log $K_1$–log $K_3$ predominantly refer to the phenolate protonations, while log $K_5$ and log $K_4$ reflect the basicity of primary and secondary amino groups, respectively.

Fig. 2 shows the relative distribution of the seven differently protonated macrospecies, as a function of pH (vancomycin proton-speciation). All the mono- to pentaprotonated macrospecies are composites of microspecies (protonation isomers), of which some can be the mechanistically reactive forms.

An overall zero charge state of the molecule on the binding of four protons is reached at pH 8.30 (isoelectric point). At pH 7.4 the average charge of vancomycin is +0.67. Accordingly, at physiological pH predominantly penta- and tetraprotonated species exist.

The microspeciation of vancomycin by NMR spectroscopy is in progress.

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