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Kinetic and equilibrium characterization of interactions between glycopeptide antibiotics and sodium carboxymethyl starch

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Abstract

The binding of two glycopeptide antibiotics, vancomycin and ramoplanin, to sodium carboxymethyl starch (NaCMS) has been studied. An ultrafiltration technique was employed with spectrophotometric detection to measure unbound drug fractions. Both drugs exhibited reversible, saturable, Langmuir-type binding over limited ranges of concentration and pH. Binding was studied at 22, 37 and 50°C to yield binding constants, the number of sites and appropriate thermodynamic parameters, using a generalized Scatchard model. Binding data were analyzed using least-squares nonlinear regression. Results suggest electrostatic attraction as the predominant mechanism for vancomycin and ramoplanin binding to NaCMS. Binding is exothermic for both drugs and appears to be enthalpy-driven for vancomycin. The binding of ramoplanin, however, is favored by enthalpy and entropy contributions. By contrast, these drugs exhibit strikingly different entropy changes upon binding to NaCMS. These results are interpreted in light of solution conformation data for both drugs. Controlled kinetic studies were also conducted to measure the rates of these interactions to determine possible therapeutic implications.

Keywords: Adsorption; Glycopeptide antibiotic; Vancomycin; Ramoplanin; Sodium carboxymethyl starch; Sodium starch glycolate

1. Introduction

Vancomycin and ramoplanin are glycopeptide antibiotics used to treat local gastrointestinal bacterial infections. In most cases, vancomycin is used to eradicate gram-positive bacteria that has

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been shown to be resistant to traditional antibiotics (e.g. methicillin-resistant *Staphylococcus aureus*) (Daschner and Kropec, 1995). Due to its different mechanism of action, ramoplanin has been investigated in recent years to treat vancomycin-resistant gastrointestinal bacteremia (Collins et al., 1993).

During formulation of an oral ramoplanin dosage form, an interaction was discovered between ramoplanin and sodium carboxymethyl starch (NaCMS), also known as sodium starch glycolate (Explotab®, Primojel®) (Claudius and Neau, 1994). Additional investigation revealed a similar interaction between vancomycin and NaCMS. It was hypothesized that these interactions were the result of an electrostatic attraction mechanism. In water, both drugs possess positively-charged free-amino functional groups that would be attracted to carboxylate groups on NaCMS, thus providing the driving force for adsorption.

In considering interactions of drugs with excipients, most investigators have studied these processes only by use of equilibrium binding studies (Rivera and Ghodbane, 1994; Pesonen et al., 1995). Although this is an important means to characterize interactions, and equilibrium binding studies are described herein, the relevance of these interactions to therapeutics is best addressed by time-dependent (i.e. kinetic) studies of the interactions. In the case of orally delivered drugs, the rate and extent to which drugs interact with excipients will determine whether the interaction will impact absorption, distribution, metabolism and elimination of drugs intended for systemic action. Although the drugs presently studied are not absorbed systemically when given orally and are intended only for local action, interactions with NaCMS could potentially affect bactericidal activity and hydrolytic or enzymatic metabolism in the gastrointestinal tract.

The purpose of this study was to characterize the kinetic nature of these interactions by estimating the rate constant and order associated with the interactions between NaCMS and glycopeptide antibiotics; specifically, vancomycin and ramoplanin. In addition, equilibrium binding studies were conducted to estimate binding constants and other thermodynamic parameters to elicit information on the binding mechanism and driving forces involved in these interactions.

2. Materials and methods

Vancomycin (ICN Biochemicals, Los Angeles, CA), ramoplanin (Gruppo Lepetit SpA, Brindisi, Italy) and sodium carboxymethyl starch (Explotab®, Edward Mendell Company, Patterson, NY) were used without further purification. Vancomycin was obtained as the monohydrochloride salt form (MW = 1485.67). Ramoplanin was obtained in the dihydrochloride salt form. Ramoplanin has been characterized as a mixture of three homologs (Parenti et al., 1990). Typically, the A2 component accounts for at least 75% of ramoplanin on a molar basis. Given this, our study was conducted using physicochemical data for the A2 dihydrochloride component (MW = 2627.01). The structures of vancomycin and ramoplanin (component A2) are shown in Figs. 1 and 2, respectively.

Standard curves were generated for vancomycin at 280 nm (λ_{max}) from 25 to 300 μg/ml and for ramoplanin at 268 nm (λ_{max}) from 8 to 112 μg/ml, using a 10 mm cell in a diode array spectrophotometer (Hewlett Packard 8451A, Cupertino, CA). Absorbance measurements for ramoplanin at 250 and 500 μg/ml were made using a 1 mm cell. These data were corrected for differences in cell path length prior to data analysis.

The vancomycin and ramoplanin concentra-

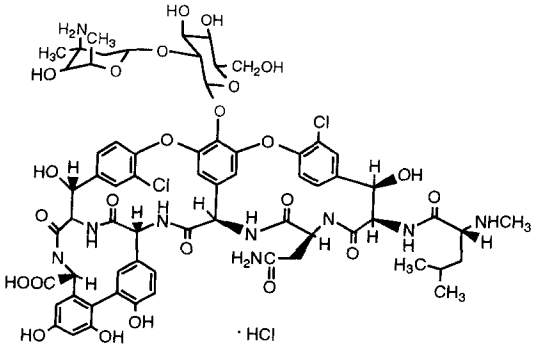

Fig. 1. Vancomycin hydrochloride.

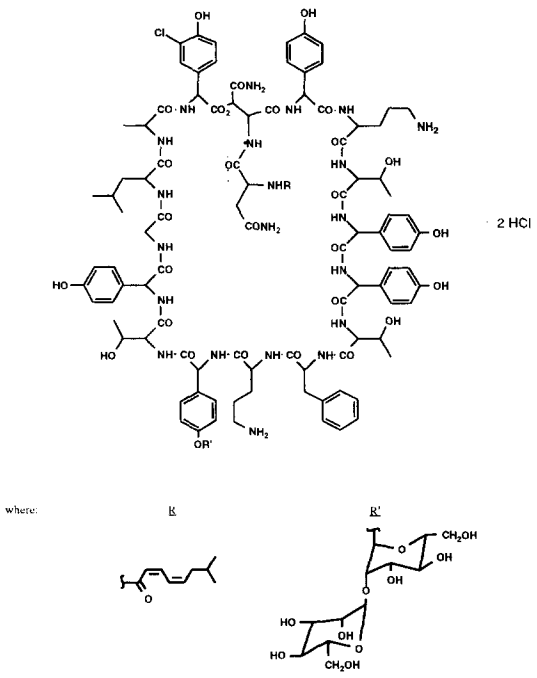

Fig. 2. Ramoplanin A2 dihydrochloride.

tions studied were similar to capsule strengths available in marketed and clinical dosage forms. Vancomycin and ramoplanin have minimum oral therapeutic doses of 125 mg four times daily (Physician's Desk Reference®, 1992) for localized gastrointestinal infections (e.g. staphylococcal enterocolitis). This would result in an equilibrium concentration of approximately 140 μg/ml in a United States Pharmacopeia (USP) dissolution testing model, based on 900 ml of dissolution medium. Correlating in vitro concentrations with observed kinetic results could then be used to predict in vivo performance. The typical levels of NaCMS, when used as a potent disintegrant in oral dosage forms, are 1–10% (w/w). The corresponding concentrations of NaCMS in this same model, then, would be approximately 10–100 μg/ml, based on an average tablet mass of 1 g.

2.1. Kinetic studies

Duplicate samples of each drug solution were prepared using purified water, USP, and placed in a 500 ml jacketed glass reaction vessel. The temperature of the interaction medium was maintained by a water bath and studies were controlled at 15, 25 and 37°C to determine the effect of temperature on kinetic parameters. Solutions were well-stirred by a $\frac{3}{4}$ inch octagonal stir bar on a magnetic stir plate. The effect of stirring rate was studied under the experimental conditions described above to determine whether or not convective effects were rate limiting in these studies. Solutions were pumped by a peristaltic pump through a 10 μm in-line filter to a Hewlett Packard 8451A diode array spectrophotometer with a 10 mm flow-through cell after achieving constant temperature (±0.5°C). NaCMS was added 30 s after establishing a stable absorbance reading for the glycopeptide, and absorbance was recorded every 10 s for 5 min. Upon exiting the spectrophotometer, the solution was returned to the reaction vessel, thus maintaining the bulk solution at a constant volume. The total analytical system volume, consisting of tubing, cell and pump dead volume, was 6.6 ml, a negligible volume in comparison to the 500 ml of the reaction vessel. The pump flow rate was 7.8 ml/min. The lag time, defined as the ratio of the analytical system volume to the pump flow rate, was accounted for in treatment of the data.

Following each measurement, all surfaces were rinsed thoroughly with purified water and wiped clean to remove residual NaCMS, which is extremely tacky and adheres to most surfaces when wetted. All surfaces were visually inspected for residual NaCMS. The outlet probe and filter were rinsed with purified water for at least 3 min discharging to waste. Absorbance of this solution was measured to ensure that all drug had been removed and that a negligible absorbance reading was achieved. The system was then pumped dry to remove any water and all probes were returned to the reaction vessel for use in subsequent measurements. This procedure was followed between any two experimental procedures to eliminate carryover of drug or NaCMS.

2.2. Binding studies

Preliminary results revealed that ramoplanin binds to Spectra/Por® 1 dialysis tubing (Spectrum

Medical Industries, Houston, TX) in water at 22°C. Consequently, alternate methods were investigated to evaluate the binding of vancomycin and ramoplanin to NaCMS. A centrifugation technique was inappropriate because NaCMS would slough off the inside wall of the centrifuge tube after centrifugation ($2000 \times g$ for 10 min). Since equilibrium dialysis and ultrafiltration techniques are considered thermodynamically equivalent (Chignell, 1977), binding studies were conducted by filtration using 25 mm acrylic copolymer Acrodisc® Versapor® syringe filters with a 0.45 μm pore size (Gelman Sciences, Ann Arbor, MI). All filters were saturated with vancomycin or ramoplanin drug solutions by measuring the absorbance of these filtered solutions until a constant absorbance reading was achieved, thus indicating saturation of the filter. The saturation of these filters eliminated the effect of binding of the drug to the filter on adsorption data.

Binding studies were conducted at 22, 37 and 50°C using duplicate samples of 500 ml solutions of drug in a USP Type II dissolution apparatus with paddles rotating at 100 rpm. Once NaCMS was added to the solution, absorbance readings were recorded at least every 2 h to determine the time for binding to reach equilibrium. The equilibrium data were analyzed using a nonlinear least-squares regression program (Systat®, SYSTAT, Evanston, IL) based on an iterative method to determine binding constants and number of sites.

Ionic strength studies were conducted in water by adjusting ionic strength with sodium chloride. Studies demonstrating the effects of pH on the interaction were conducted using a 10 mM phosphate/acetate buffer at constant ionic strength. Samples were shaken overnight at room temperature (nominally 22°C), filtered and then analyzed against proper controls the following day.

3. Results and discussion

Standard curves for vancomycin and ramoplanin were demonstrated to be linear ($r^2 > 0.999$) over the range of concentrations studied. From these data, the molar absorptivity of vancomycin at 280 nm is estimated to be 0.55×10^4 l/mol per cm and the molar absorptivity of ramoplanin at 268 nm is estimated to be 2.79×10^4 l/mol per cm.

The data presented in Fig. 3 show that the rate and extent of adsorption can be affected by the stirring speed of the magnetic stir bar under the conditions studied. The rate and extent of adsorption, however, is consistent at higher stirring speeds (> 600 rpm). This suggests that an aqueous boundary layer at the solid–liquid interface is limiting the rate and extent of adsorption of ramoplanin onto NaCMS at slower stirring speeds. Therefore, all kinetic studies were conducted at 1200 rpm to ensure that transport across the boundary layer was not rate limiting.

The kinetic profile of the interaction between the glycopeptides and NaCMS in water at 37°C is shown in Fig. 4. An algorithm was designed to retain the initial linear portion of the kinetic data by maximizing the Pearson correlation coefficient of the initial data points. Using these data, the initial rate of these interactions was determined by fitting the kinetic data to the following general reaction rate model:

Rate of interaction

$$= -\mathrm{d}(c_\mathrm{d})/\mathrm{d}t = k \cdot (c_\mathrm{d})^a \cdot (c_\mathrm{cms})^b = k_\mathrm{obs} \qquad (1)$$

where

$k_\mathrm{obs} \equiv$ observed rate constant (μg/ml per min)

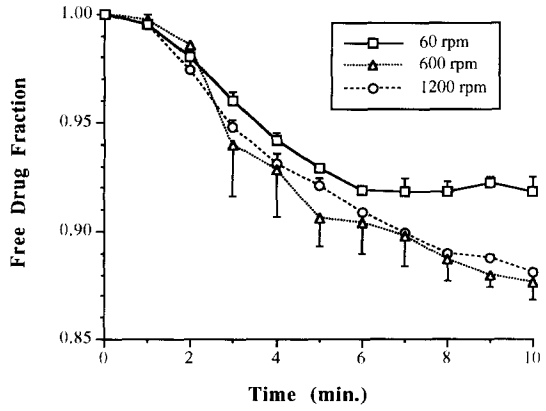

Fig. 3. Effect of stirring speed on the interaction of ramoplanin with NaCMS (10 μg/ml).

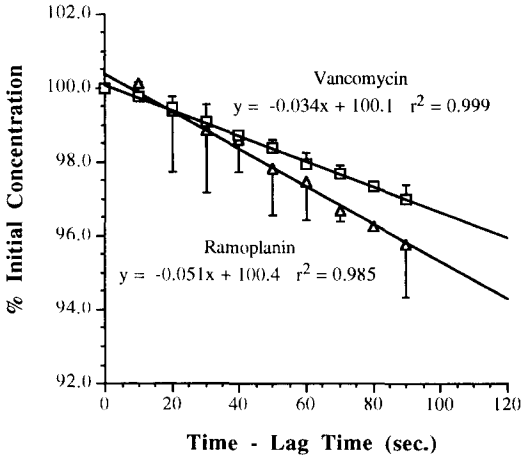

Fig. 4. Kinetic profile of glycopeptide interactions with NaCMS (10 μg/ml) at 37°C in water.

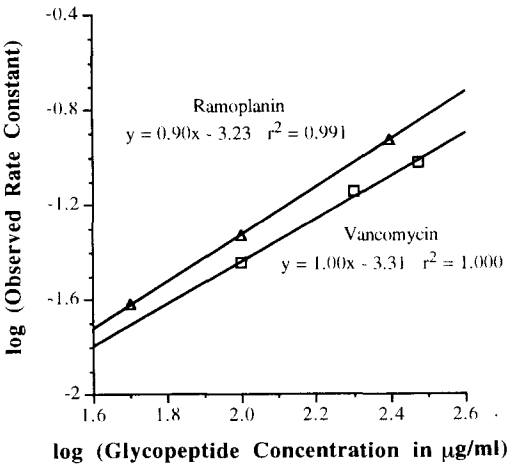

Fig. 5. Effect of glycopeptide concentration on the observed interaction rate constant at 37°C.

$k \equiv$ interaction rate constant (ml/μg per min)
$(c_d) \equiv$ drug concentration (μg/ml)
$(c_{cms}) \equiv$ NaCMS concentration (μg/ml)
$a \equiv$ interaction order with respect to drug
$b \equiv$ interaction order with respect to NaCMS

The rate constant, individual and overall orders of these interactions were determined by the method of initial slopes (Atkins, 1982). The method of initial slopes requires logarithmic transformation of Eq. (1), resulting in the following form:

$$\log k_{obs} = \log k + a \log(c_d) + b \log(c_{cms}) \quad (2)$$

Therefore, the plots of $\log k_{obs}$ versus $\log(c_d)$ and $\log k_{obs}$ versus $\log(c_{cms})$ in Figs. 5 and 6 yield the interaction rate constant, k, and the individual order of the interaction with respect to glycopeptide and NaCMS concentration (a, b), as shown in Table 1. The interaction rate constant and orders are calculated from simultaneous solution of regression equations in Figs. 5 and 6. The interaction orders, a and b, are obtained from the slopes of regression equations in Figs. 5 and 6, respectively. The interaction rate constant, k, is then calculated from the slopes and intercepts of Figs. 5 and 6.

Results indicate that these interactions are second-order overall, and first-order with respect to vancomycin and ramoplanin concentrations. The magnitudes of the interaction rate constants are similar to dissolution rate constants for both vancomycin and ramoplanin, as shown in Table 2. These results indicate that the rate of binding to NaCMS is similar in magnitude to the dissolution rate of the drug, assuming that the dissolution of the drug is a kinetically controlled process. Thus, the time-dependent nature of the adsorption may impact therapeutic treatment with these drugs in the gastrointestinal tract.

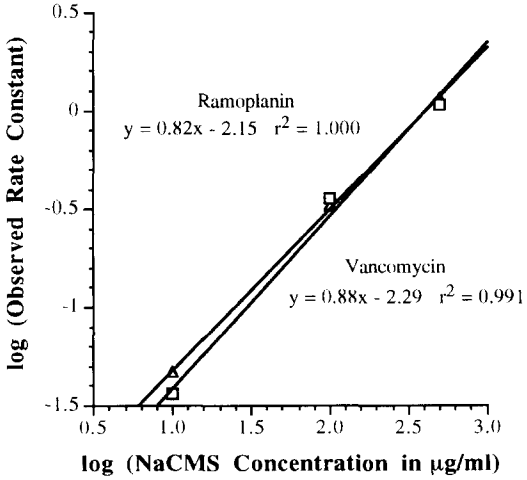

Fig. 6. Effect of NaCMS concentration on the observed interaction rate constant at 37°C.

Table 1
Kinetic parameters of interaction between glycopeptide antibiotics and NaCMS at 37°C

	k (ml/μg per h)	a	b
Vancomycin	0.227 ± 0.003	1.0	0.9
Ramoplanin	0.360 ± 0.016	0.9	0.8

In equilibrium binding studies, the time to reach equilibrium was concentration-dependent with respect to both drug and NaCMS concentration and was generally achieved in less than 12 h at all temperatures studied. Equilibrium binding constants were estimated at these temperatures using the following generalized model (Scatchard, 1949) which accounts for multiple classes of independent binding sites with different binding affinities:

$$R = \sum_{i=1}^{j} n_i K_i[L]/(1 + K_i[L]) \quad (3)$$

where R is the fraction of total sites occupied, n_i is the number of binding sites of class i per molecule, K_i is the binding constant associated with class i sites, and $[L]$ is the free drug concentration. Binding constants and number of sites were estimated from the equilibrium binding data by using a nonlinear least-squares regression program as described earlier.

Certain authors (Upadrashta and Wurster, 1989) have expressed concern regarding the use of linearized forms of Eq. (3), such as the Klotz or double-reciprocal plot. These authors contend that the inverse transformation of Eq. (3) distorts the true variability of the data by disproportionately weighting the most variable individual data points. As a result, nonlinear regression was chosen as the primary method for analysis of the data.

Table 2
Comparison of dissolution and observed interaction rate constants for glycopeptide interactions with NaCMS at 37°C

	k_{obs} (μg/ml per min)	k^*_{diss} (μg/ml per min)
Vancomycin	2.04 ± 0.01	3.70
Ramoplanin	3.06 ± 0.03	2.96

* k_{diss} is calculated based on 80% drug released in initial 30 min (vancomycin 125 mg, ramoplanin 100 mg capsules).

Statistical analysis revealed similar analysis of variance (ANOVA) results for one- and two-site binding models, indicating that a one-site Scatchard model adequately describes the data under the conditions studied for both vancomycin and ramoplanin binding to NaCMS. Using a one-site binding model, binding constants and the number of sites were estimated and are presented in Table 3. These results indicate that ramoplanin binds to NaCMS with greater affinity than does vancomycin. This is confirmed in Fig. 7a and b which demonstrate the Langmuir-type binding of vancomycin and ramoplanin to NaCMS.

The change in enthalpy between bound and unbound states, ΔH°, is obtained by plotting the binding constants obtained at different temperatures versus reciprocal temperature (i.e. van't Hoff plot) as in Fig. 8, according to the following equation:

$$\mathrm{d}(\ln K_{eq})/\mathrm{d}(1/T) = -\Delta H^\circ/R \quad (4)$$

The change in entropy between bound and unbound states, ΔS°, is then obtained from the following:

$$\Delta S^\circ = \frac{\Delta H^\circ - \Delta G^\circ}{T} \quad (5)$$

where $\Delta G^\circ = -RT \ln K_{eq}$, and K_{eq} is in M^{-1}. These thermodynamic parameters are presented in Table 4 for vancomycin and ramoplanin binding to NaCMS at 37°C.

Negative ΔG° values, of course, indicate the spontaneity of the adsorption process. The negative change in enthalpy indicates an exothermic process over the temperature range studied (22–50°C) and is confirmed in Table 3 by the decreased binding constants observed at higher temperatures. The magnitude of the change in enthalpy as vancomycin binds to NaCMS indicates an enthalpy-driven process and is substantiated by a negative change in entropy. The binding of ramoplanin, however, is favored by both enthalpy and entropy contributions. The striking difference is the change in entropy estimated for each drug. For vancomycin, it is negative and indicates an increase in system order upon ad-

Table 3
Binding constants (K_{eq}) and number of sites (n) for glycopeptide interactions with NaCMS (M.S.E. = mean squared error)

	22°C			37°C			50°C		
	n	K_{eq} (μM^{-1})	M.S.E.	n	K_{eq} (μM^{-1})	M.S.E.	n	K_{eq} (μM^{-1})	M.S.E.
Vancomycin	1.04	0.156	0.009	1.33	0.050	0.009	1.27	0.034	0.002
Ramoplanin	0.94	0.886	0.002	1.14	0.801	0.002	1.21	0.498	0.006

sorption to NaCMS. This makes intuitive sense given that positioning of the vancomycin molecule is confined after adsorption to NaCMS. By contrast, the change in entropy for ramoplanin is positive, which indicates a decrease in system order upon adsorption. This is an apparent discrepancy that exists between two structurally similar molecules but which can be explained in light of the conformation of these drugs in solution.

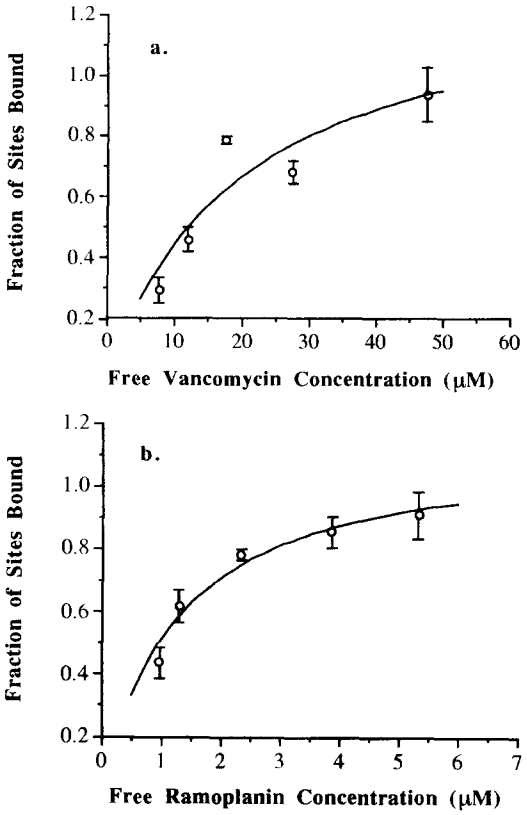

Fig. 7. (a) Binding isotherm for vancomycin adsorption onto NaCMS (10 μg/ml) at 37°C; (b) binding isotherm for ramoplanin adsorption onto NaCMS (10 μg/ml) at 37°C.

The solution conformation of vancomycin has been studied using nuclear magnetic resonance (NMR) spectroscopy (Groves et al., 1994). Results demonstrate that vancomycin exists in solution as asymmetric homodimers resulting from interaction of the sugar residues. To elicit its bactericidal action, the methylated amino sugar on vancomycin plays a key role in binding with the bacterial cell wall peptide fragment, di-*N*-acetyl-L-Lys-D-Ala-D-Ala (Beauregard et al., 1995). This binding process has been shown to occur through cooperativity of nonpolar and ionic forces (Cristofaro et al., 1995). The binding of vancomycin to NaCMS also reveals ionic contributions to the interaction. The increase of solution ionic strength causes a decrease in binding, as shown in Fig. 9, thus indicating electrostatic attraction as the main driving force for adsorption. The essential elimination of adsorption below pH 4, as shown in Fig. 10, implies that the carboxylate moiety on NaCMS is involved in binding

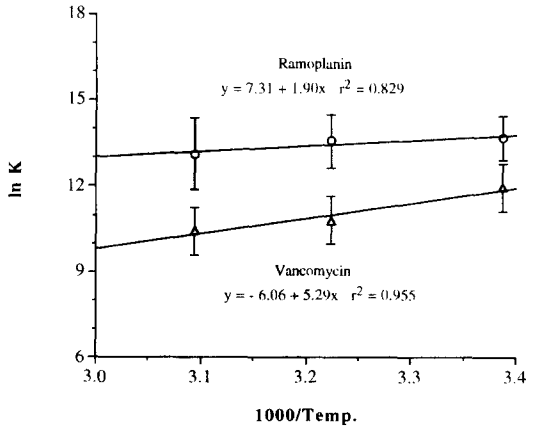

Fig. 8. van't Hoff plot of vancomycin and ramoplanin adsorption onto NaCMS.

Table 4
Thermodynamic parameters of glycopeptide interactions with NaCMS at 37°C

	$\Delta G°$ (kcal/mol)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/(mol·K))
Vancomycin	-6.66 ± 0.47	-10.51 ± 0.73	-12.42 ± 0.87
Ramoplanin	-8.38 ± 0.57	-3.78 ± 0.26	$+14.82 \pm 1.02$

with the methylated amino sugar or the methylleucine amine on vancomycin. The acid–base properties for the ionizable groups of van comycin have been determined at 25°C (Takács-Novák et al., 1993). The pK_a values for the primary and the secondary amines were estimated to be 7.75 and 8.89, respectively. These ionization constants support the findings presented here indicating an electrostatic attraction mechanism between the free amino groups on vancomycin and the carboxylate groups on NaCMS. The vancomycin carboxylate pK_a was determined by Takács-Novák et al. to be 2.18. This carboxylate group is far removed from either of the amines on vancomycin and would not be expected to interfere with the proposed binding mechanism.

The thermodynamics of binding of ramoplanin to NaCMS is considerably different from that of vancomycin. The positive change in entropy favors ramoplanin binding to NaCMS and indicates an overall decrease in system order. One plausible explanation is that ramoplanin is more ordered when solvated than after adsorption onto NaCMS. The solution conformation of ramoplanin has been determined and supports this hypothesis (Maplestone et al., 1993). NMR data show that ramoplanin adopts a stable secondary structure in which the amide NHs are protected from the solvent through intramolecular hydrogen bonding. The beta-sheet conformation that results is characterized by two antiparallel strands interconnected by seven intramolecular hydrogen bonds and two reverse turns. The adsorption of ramoplanin onto NaCMS could result in a decrease of order as a consequence of the disruption of this highly ordered solution conformation. The effects of ionic strength and pH on the binding of ramoplanin to NaCMS are consistent with those effects observed with vancomycin binding to NaCMS, as seen in Figs. 9 and 10. These data suggest that the carboxylate moiety on NaCMS is involved in binding with the free amino ornithine residues of ramoplanin, again indicating electrostatic attraction as the driving force for these interaction phenomena.

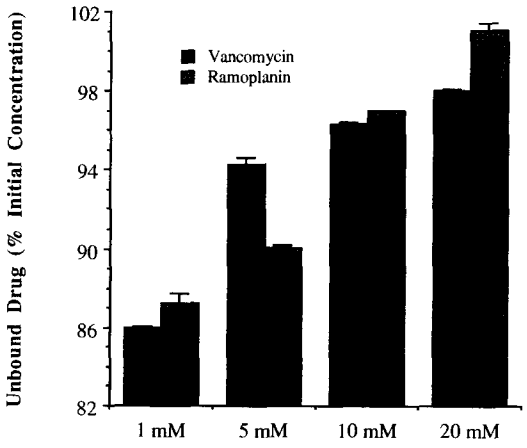

Fig. 9. Effect of ionic strength on the interaction between glycopeptides and NaCMS at 22°C.

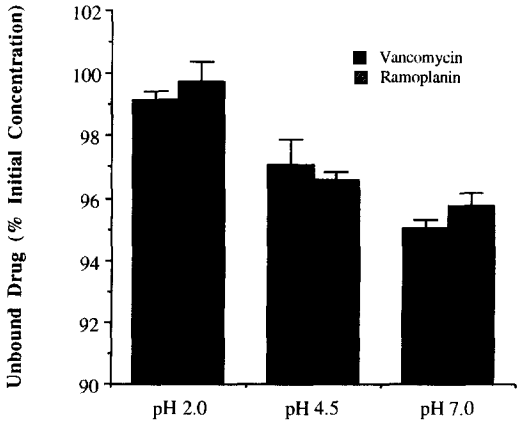

Fig. 10. Effect of pH on the interaction of glycopeptides and NaCMS in 10 mM buffer at 22°C.

4. Conclusions

The interactions between NaCMS and vancomycin or ramoplanin have been characterized by conducting kinetic and equilibrium binding studies. Results from kinetic studies indicate that the rates of these interactions are second-order overall, and first-order with respect to vancomycin and ramoplanin concentrations. The interaction rate constants describing these interactions are on the same order of magnitude as the dissolution rate constants for both drugs, indicating possible therapeutic implications. Equilibrium binding studies demonstrated that the binding of vancomycin is enthalpy-driven, whereas ramoplanin binds to NaCMS through enthalpy and entropy contributions. Vancomycin and ramoplanin exhibit opposite changes in entropy upon adsorbing to NaCMS. In light of previous research, these differences in the change in entropy are most likely a direct reflection of the conformation of each drug in solution. Ionic strength and pH studies support electrostatic attraction between glycopeptide free amino groups and carboxylate groups on NaCMS as the primary driving force for adsorption.

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References

Atkins, P.W., *Physical Chemistry*, 2nd Edn, W.H. Freeman and Company, San Francisco, 1982, p. 926.

Beauregard, D.A., Williams, D.H., Gwynn, M.N. and Knowles, D.J., Dimerization and membrane anchors in extracellular targeting of vancomycin group antibiotics. *Antimicrob. Agents Chemother.*, 39 (1995) 781–785.

Chignell, C.F., Protein binding. In Garrett, E.R. and Hirtz, T.L. (Eds), *Drug Fate and Metabolism: Methods and Techniques*, Vol. 1, Marcel Dekker, New York, 1977, p. 187.

Claudius, J.S. and Neau, S.H., Solution interaction of a polypeptide antibiotic with starch-based excipients. *Pharm. Res.*, 11 (1994) S293.

Collins, L.A., Eliopoulos, G.M., Wennersten, C.B., Ferraro, M.J. and Moellering, R.C., In vitro activity of ramoplanin against vancomycin-resistant gram-positive organisms. *Antimicrob. Agents Chemother.*, 37 (1993) 1364–1366.

Cristofaro, M.F., Beauregard, D.A., Yan, H., Osborn, N.J. and Williams, D.H., Cooperativity between non-polar and ionic forces in the binding of bacterial cell wall analogues by vancomycin in aqueous solution. *J. Antibiot.*, 48 (1995) 805–810.

Daschner, F.D. and Kropec, A., Glycopeptides in the treatment of staphylococcal infections (Review). *Eur. J. Clin. Microbiol. Infect. Dis.*, 14 (Suppl. 1) (1995) S12–S17.

Groves, P., Searle, M.S., Mackay, J.P. and Williams, D.H., The structure of an asymmetric dimer relevant to the mode of action of the glycopeptide antibiotics. *Structure*, 2 (1994) 747–754.

Maplestone, R.A., Cox, J.P. and Williams, D.H., Retention of native-like structure in an acyclic counterpart of a beta-sheet antibiotic. *FEBS Lett.*, 326 (1993) 95–100.

Parenti, F., Ciabatti, R., Cavalleri, B. and Kettenring, J., Ramoplanin: a review of its discovery and its chemistry. *Drugs Exp. Clin. Res.*, 16 (1990) 451–455.

Pesonen, T., Kanerva, H., Hirvonen, J., Nuuja, T. and Pohjola, J., Incompatibilities between chlorhexidine diacetate and some tablet excipients. *Drug Dev. Ind. Pharm.*, 21 (1995) 747–752.

Physician's Desk Reference®, 46th Edn, Medical Economics Data, Montvale, NJ, 1992, pp. 1304–1305.

Rivera, S.L. and Ghodbane, S., In vitro adsorption–desorption of famotidine on microcrystalline cellulose. *Int. J. Pharm.*, 108 (1994) 31–38.

Scatchard, G., The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.*, 51 (1949) 660–672.

Takács-Novák, K., Noszál, B., Tókés-Kövesdi, M. and Szász, G., Acid–base properties and proton-speciation of vancomycin. *Int. J. Pharm.*, 89 (1993) 261–263.

Upadrashta, S.M. and Wurster, D.E., Equilibrium binding studies of the interaction between anthralin and bovine serum albumin. *Int. J. Pharm.*, 49 (1989) 103–108.