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International Journal of Mass Spectrometry 188 (1999) 63–85

International Journal of Mass Spectrometry

Collision-induced dissociation of noncovalent complexes between vancomycin antibiotics and peptide ligand stereoisomers: evidence for molecular recognition in the gas phase

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Received 14 July 1998; accepted 18 August 1998

Abstract

In solution, the antibiotics of the vancomycin group bind stereospecifically to peptides with the C-terminal sequence: –L-Lys–D-Ala–D-Ala. Substitution by a L-Ala at either of the two C-terminal residues causes a dramatic decrease in the binding affinity to the antibiotics. This solution behavior is clearly reflected in electrospray ionization (ESI) mass spectra obtained from equimolar mixtures of an antibiotic, an isotopically labelled peptide ligand and an unlabelled peptide stereoisomer. Using collision-induced dissociation (CID) we have probed the gas phase stability of isomeric (1:1) noncovalent complexes formed between vancomycin and tripeptide stereoisomers. In negative ion mode the CID results show that a complex formed between vancomycin and a –L-Ala–L-Ala ligand fragments more readily than a complex formed between vancomycin and a –D-Ala–D-Ala ligand. This difference in gas phase stability is in agreement with what would be expected if the noncovalent complexes had retained their structural specific interactions from solution to gas phase. In positive ion mode no significant difference in the gas phase stabilities of the isomeric complexes could be observed. We attribute this similarity in gas phase stability between isomeric positively-charged complexes to a protonation of the C-terminus of the peptide ligand which destroys the specific interaction between antibiotic and peptide ligand. Molecular recognition phenomena in the gas phase were investigated by CID of mixed cluster ions consisting of an antibiotic, a –L-Ala peptide, a –D-Ala stereoisomer, one ligand isotopically labelled. Upon CID of the negatively charged mixed cluster ions a stereoselective loss of the assumed "nonspecifically" bound –L-Ala ligand was observed. (Int J Mass Spectrom 188 (1999) 63–85) © 1999 Elsevier Science B.V.

Keywords: Mass spectrometry; Noncovalent complexes; Glycopeptide antibiotics; Molecular recognition; Electrospray ionization; Collision-induced dissociation

1. Introduction

Electrospray ionization mass spectrometry (ESI-MS) is becoming a recognized method for studying noncovalent complexes in solution and the properties of a variety of biological noncovalent macromolecular complexes have been observed with this method [1–3]. It is often assumed that the noncovalent complex ions which are formed in the gas phase by electrospray ionization at least partially retain their solution structure [1,4–6]. The observation that mul-

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Dedicated to Brian Green on the occasion of his 65th birthday.

PII S1387-3806(98)14282-3

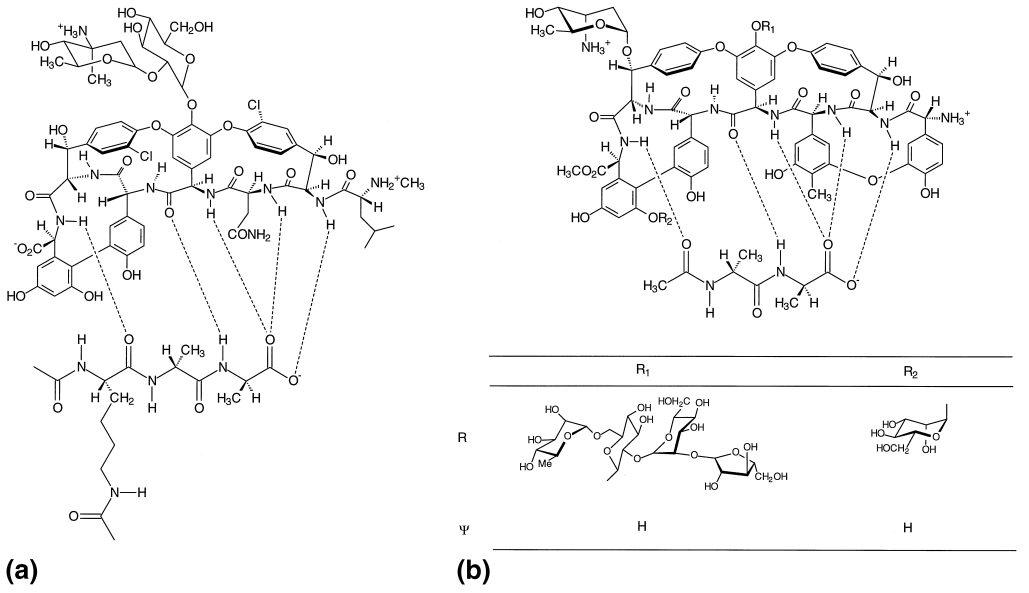


Fig. 1. (a) Noncovalent complex formed between vancomycin and the tripeptide cell wall analogue Ac_2–L-Lys–D-Ala–D-Ala. Dotted lines indicate hydrogen bonds. Net charge of complex is zero. (b) Noncovalent complex formed between an antibiotic (ristocetin or pseudoaglycoristocetin) and the dipeptide cell wall analogue Ac–D-Ala–D-Ala. Dotted lines indicate hydrogen bonds. Net charge of complex is +1.

tiply charged protein ions appear to have distinguishable conformations in the gas phase [7] suggests that some correlation between gas phase and solution conformation may exist but without a direct link between the solution phase structure and gas phase structure the question remains as to how closely the gas phase and solution phase structures are related.

Complexation between various members of the vancomycin group antibiotics and peptide ligands in solution has been studied by ESI-MS [8–10]. The binding of peptide ligands to this class of antibiotics have been extensively studied by nuclear magnetic resonance (NMR) [11–14] and other spectroscopic techniques [15–19]. Inhibition of bacterial cell-wall growth by this class of antibiotics is based on binding of the antibiotics to cell-wall precursors with the C-terminal sequence –D-Ala–D-Ala [20]. From model studies this binding is known to involve several intermolecular hydrogen bonds as well as hydrophobic interactions between the two alanine methyl groups of the peptide ligands and regions of the benzene moieties of the antibiotics [11,15,21]. In Fig. 1(a) and (b) is shown the intermolecular hydrogen bonds in two such complexes between two antibiotics and two peptide ligands.

The structural features of the peptide ligands which are most critical for the binding are the two C-terminal D-Ala residues [15,22]. Substitution by an L-amino acid at either of the two C-terminal residues impairs complex formation and results in a dramatic decrease in the ligand binding affinity to vancomycin. Thus complex formation in solution is critically dependent on the chirality of the residues of the ligand. This solution behaviour is clearly reflected in the positive ion ESI mass spectrum of an equimolar mixture of vancomycin, Ac_2–L-Lys–D-Ala–D-Ala and d_6-Ac_2–L-Lys–L-Ala–L-Ala. Only the complex formed between vancomycin and Ac_2–L-Lys–D-Ala–D-Ala can be observed in the ESI mass spectrum [23]. When a solution containing vancomycin and the nonspecific d_6-Ac_2–L-Lys–L-Ala–L-Ala ligand is analyzed by ESI-MS, a peak corresponding to a noncovalent complex formed between the peptide ligand and vancomycin can be observed. However, the ion abun-

dance of this complex is markedly reduced compared to the abundance of the complex formed between vancomycin and the Ac_2–L-Lys–D-Ala–D-Ala ligand.

The purpose of the present work is to examine the extent that the structurally specific interactions of the antibiotic–peptide complex, which are present in solution, are retained when the complex is examined in the gas phase. It is assumed that if the stability of the complex in the gas phase depends on the same structural features as the stability in solution, then the structural features responsible for complex formation in solution are maintained in the gas phase. In particular, the influence of the stereochemistry of the peptide on the stability has been examined.

The method we have used to investigate the complexes in the gas phase is collision-induced dissociation (CID) in tandem mass spectra experiments. The stability of the complexes have been investigated in CID experiments on 1:1 complexes (A + L1) consisting of one antibiotic molecule (A) and one peptide ligand (L1) and in competitive reaction experiments on 1:2 complexes (A + L1 + L2) consisting of one antibiotic molecule (A) and two peptide ligands (L1 and L2). The antibiotics are known to have a single peptide binding site but the 1:2 complexes (mixed cluster ions) can be formed from solutions of sufficiently high concentrations of the peptides.

The antibiotics included in the work are vancomycin (V) and ristocetin (R) as well as four derivatives, aglycovancomycin (aV), aglycovancomycinmethylester ($aVOCH_3$), and pseudoaglycoristocetin (Ψ), in which saccharide substituents which are not directly involved in the binding of peptides in solution have been removed. The peptides are given in the list below and are either tripeptides or dipeptides.

2. Experimental

2.1. Antibiotics

Vancomycin hydrochloride and ristocetin sulfate were kindly donated by Dumex (Copenhagen, Denmark) and Alpharma (Oslo, Norway), respectively. These compounds were used without further purification. Aglycovancomycin was prepared by acid hydrolysis of vancomycin [24]. Aglycovancomycinmethylester ($aVOCH_3$) was prepared by acid catalyzed esterification of aglycovancomycin. Acetylchloride (480 μL) was carefully added to cold dry methanol (3 mL) and aglycovancomycin (0.25 mg) was dissolved herein. After 2 h at room temperature (22 °C) the reaction mixture was evaporated *in vacuo*, aglycovancomycinmethylester was redissolved in water and purified by reversed-phase (C-18) high-performance liquid chromatography (HPLC). Methylation was confirmed by ESI-MS (MH^+ m/z 1157.4) and with ESI-MS/MS in negative ion mode, where the characteristic loss of CO_2 (decarboxylation of the C-terminus) was absent. Pseudoaglycoristocetin (Ψ) was prepared by methanolysis of ristocetin [25] and purified by reversed-phase (C-18) HPLC. All solvents were HPLC grade or equivalent quality, and were used without further purification.

2.2. Peptide synthesis

The following peptides were used:

	Abbreviation	
$(CH_3–CO)_2$–D-Lys–D-Ala–D-Ala	(1)	ac_2KAA(DDD)
$(CD_3–CO)_2$–D-Lys–L-Ala–L-Ala	(2)	d_6ac_2KAA(DLL)
$(CD_3–CO)_2$–D-Lys–D-Ala–D-Ala	(3)	d_6ac_2KAA(DDD)
$(CH_3–CO)_2$–D-Lys–L-Ala–L-Ala	(4)	ac_2KAA(DLL)
$(CH_3–CO)_2$–L-Lys–L-Ala–L-Ala	(5)	ac_2KAA(LLL)
$(CD_3–CO)_2$–L-Lys–L-Ala–L-Ala	(6)	d_6ac_2KAA(LLL)
$(CH_3–CO)_2$–L-Lys–D-Ala–D-Ala	(7)	ac_2KAA(LDD)
$(CD_3–CO)$–Gly–D-Ala	(8)	$d_3$acGA(D)
$(CH_3–CO)$–Gly–D-Ala	(9)	acGA(D)
$(CD_3–CO)$–Gly–L-Ala	(10)	$d_3$acGA(L)
$(CH_3–CO)$–Gly–L-Ala	(11)	acGA(L)
$(CH_3–CO)$–D-Ala–D-Ala	(12)	acAA(DD)
$(CD_3–CO)$–L-Ala–L-Ala	(13)	$d_3$acAA(LL)
$(CD_3–CO)$–D-Ala–Gly	(14)	$d_3$acAG(D)
$(CH_3–CO)$–D-Ala–Gly	(15)	acAG(D)
$(CD_3–CO)$–L-Ala–Gly	(16)	$d_3$acAG(L)
$(CH_3–CO)$–L-Ala–Gly	(17)	acAG(L)
$(CD_3–CO)$–D-Ala–D-Ala–D-Ala	(18)	$d_3$acAAA(DDD)
$(CH_3–CO)$–L-Ala–L-Ala–L-Ala	(19)	acAAA(LLL)

Peptides 1–4 were elongated by batch solid phase peptide synthesis at a 0.14 mmol scale using a standard Fmoc protocol [26]. The synthesis of peptide

1 and 3 was performed on a Fmoc–D-Ala–Wang resin (substitution 0.50 mmol/g; Novabiochem, Läufelfingen, Switzerland). A Fmoc-L-Ala-PAL-PEG-PS resin (substitution 0.22 mmol/g; PerSeptive Biosystems, Watford Herts, Great Britain) was used for the synthesis of peptides 2 and 4. Fmoc–L-Ala–OH (Neosystems Laboratoire, Strasbourg, France) or Fmoc–D-Ala–OH (Novabiochem) and Fmoc-D-Lys(Dde)–OH (Novabiochem) were incorporated into the resin as free acids using 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) as coupling reagent [27]. Protected amino acids and TBTU were dissolved in a solution of 8% (v/v) diisopropylethylamine (DIEA) in dimethylformamide (DMF) prior to coupling. The reaction was incubated at room temperature for 60 min before washing with DMF and Fmoc removal with 20% (v/v) piperidine in DMF. Side chain removal of the Dde protecting group was performed by treatment of the peptide resin with 2% hydrazine monohydrate in DMF [28]. Acetylation of peptides 1 and 4 was performed on 0.7 mmol of peptide resin with 20% (v/v) acetic anhydride in DMF. 0.3% (v/v) N-methylimidazole was added and the solution was gently stirred for 60 min. Deuteroacetylation of peptides 2 and 3 was achieved by incubating tetradeuteroacetic acid and O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexaflouroborate (HATU) in 4.5 mL 8% (v/v) DIEA in DMF for 60 min [29]. The reaction was monitored to completeness by the Kaiser ninhydrin test for free amino groups [30], and the reaction was repeated if necessary. Cleavage of the peptides from the resin were achieved with TFA–H_2O (95:5 v/v) and incubation at room temperature for 60 min. The peptides were precipitated by addition of ice-cold diethylether and then lyophilized. The products were characterized by reversed-phase HPLC and ESI-MS as previously described [31], and were repurified by semipreparative reversed-phase C-18 chromatography if necessary. Peptides 5 and 6 were synthesized by using conventional solution peptide synthesis. The N,N′-dibenzyloxycarbonyl–L-Lysin–N-hydroxysuccinimide ester (1 g, 1.96 mmol) was dissolved in 15 mL 1,2-dimethoxyethane and added to a solution of L-Ala-L-Ala (0.313 g, 1.96 mmol) and $NaHCO_3$ (0.336 g, 4 mmol) in water (5 mL). After 4 hours an aliquot of water (15 mL) was added and the solution acidified to pH 2 with concentrated hydrochloric acid (5 mL, 4 M). The mixture was cooled in a ice-water bath for approximately 30 min. The crystals were collected and washed on a filter with cold water (2×20 mL) and dried *in vacuo*. The protecting groups were removed by catalytic hydrogenation [32]. Following acetylation with d_6-acetic anhydride or acetic anhydride [16], the peptide was purified by ion-exchange chromatography. Purity was assessed by both mass spectrometry and polarimetry. Peptide 7 was purchased from Sigma (St Louis, MO, USA) and used without further purification. Peptides 8–19 were purchased from Sigma with free N-termini, acetylated with acetic acid anhydride or d_6-acetic acid anhydride [16], and purified by ion-exchange chromatography (strong cation exchange resin, Dowex 50).

2.3. Conditions for electrospray ionization mass spectrometry

The ESI mass spectra were recorded on a Finnigan TSQ700 triple quadrupole mass spectrometer equipped with a nanoelectrospray source [33] (Protana, Odense, Denmark). The Finnigan ESI-interface consists of a heated capillary, where the ions are desolvated before they enter the tube lens/skimmer region. A focusing octapole (API octapole) guides the ions from the skimmer region to the first quadrupole. Needle voltage was 800–900 V in positive ion mode and from −700 to −800 V in negative ion mode. All solvents were HPLC grade. The solutions for ESI-MS analysis were prepared by diluting aliquots of 2.0 mM stock solutions of antibiotic and peptide ligands with 5.0 mM ammonium acetate buffer (pH 5.1) to a concentration of 25–100 μM. In the mixed cluster ion experiments, the concentrations were typically 100 μM for the –L-Ala– peptide ligand, 50 μM for the –D-Ala– peptide ligand and 50 μM for the antibiotic. The experimental conditions for observing the intact antibiotic–peptide ligand complexes by electrospray ionization mass spectrometry were carefully controlled. In particular, the voltage difference between the tube lens and the skimmer, in addition the tem-

perature of the heated capillary required careful adjustment to provide just sufficient energy to completely desolvate the noncovalent complex ions without inducing dissociation of the complex. Typical conditions for positive ion mode were: heated capillary offset −15 V, heated capillary temperature 170 °C, tube lens voltage +50 V, API octapole offset +1.5 V, the potentials were maintained at constant settings throughout the entire mass-to-charge ratio range. In negative ion mode the potentials were reversed.

In the CID experiments, the noncovalent complex was mass selected in the first quadrupole (Q1) and then accelerated into the collision octapole (q2), which was filled with either argon or xenon. The pressure in the collision octapole was monitored by an absolute pressure transducer (MKS Baratron 627A). For the determination of the order of gas phase acidities and proton affinities of the peptide ligands argon was used as collision gas at a pressure of 0.50 mTorr. The q2 offset was +10.0 V for the negatively charged cluster ions and −10.0 V for the positively charged cluster ions. For the dissociation curves, the kinetic energy of the precursor ion was varied from 0 to 235 eV in the laboratory frame of reference (q2 offset × charge of the precursor ion). This corresponds approximately to 0–16 eV centre-of-mass collision energy, if xenon is used as the collision gas. The center-of-mass collision energy (E_{CM}) is the maximum amount of kinetic energy that can be converted into internal energy upon collisional activation under single collision conditions. E_{CM} is given by

$$E_{CM} = E_{LAB}[m_g/(m_g + m_p)]$$

where m_g is the mass of the stationary target gas, m_p is the mass of the projectile ion and E_{LAB} is the ion kinetic energy in the laboratory frame of reference. The resulting product ions were mass analyzed in the third quadrupole (Q3). In order to minimize contributions from interfering ions in CID experiments of mixed cluster ions, the width of the mass transmitting window of the first quadrupole was only 1 Thomson. This window was wider (2 Thomson), i.e. lower resolution for Q1, in the CID experiments for the dissociation efficiency curves. The dissociation efficiency curves were obtained when Q3 was operated in selected-ion-monitoring (SIM) mode. The tripeptide product ion at m/z 371 and the precursor ion at m/z 909 were thus monitored. The relative abundances of peptide product ions from CID of mixed cluster ions were determined from mass spectra averaged over 120 narrow scans. There was little variation (max. 5%) in the relative product ion abundances from five consecutive CID spectra. All other CID spectra were obtained with Q3 operating in full-scan mode. Single collision conditions were estimated by the method of Dawson [34].

3. Results

3.1. CID of (1:1) complexes between vancomycin and tripeptide stereoisomers

The relative gas phase stabilities of positively and negatively charged noncovalent complexes formed between vancomycin (V) and the four stereoisomeric peptide ligands: ac_2KAA(LDD), ac_2KAA(DDD), ac_2KAA(DLL), and ac_2KAA(LLL) were investigated by CID. In order to compare the relative energy of dissociation for the gas-phase complexes, it must be ensured that each type of noncovalent complex follows the same mechanism upon dissociation. All the dianionic noncovalent complexes $[V + ac_2KAA–2H]^{2-}$ fragment by a charge separation mechanism into a singly charged tripeptide $[ac_2KAA–H]^-$ (m/z 371) and singly charged vancomycin $[V–H]^-$ (m/z 1447) [reaction (1)]. The vancomycin product ion undergoes further fragmentation and CO_2 is lost to give the decarboxylated product ion at m/z 1403 $[V–H–CO_2]^-$ [reaction (2)].

$$[V + ac_2KAA\text{-}2H]^{2-} \rightarrow [V–H]^- + [ac_2KAA\text{-}H]^- \quad (1)$$

$$[V–H]^- \rightarrow [V–H–CO_2]^- + CO_2 \quad (2)$$

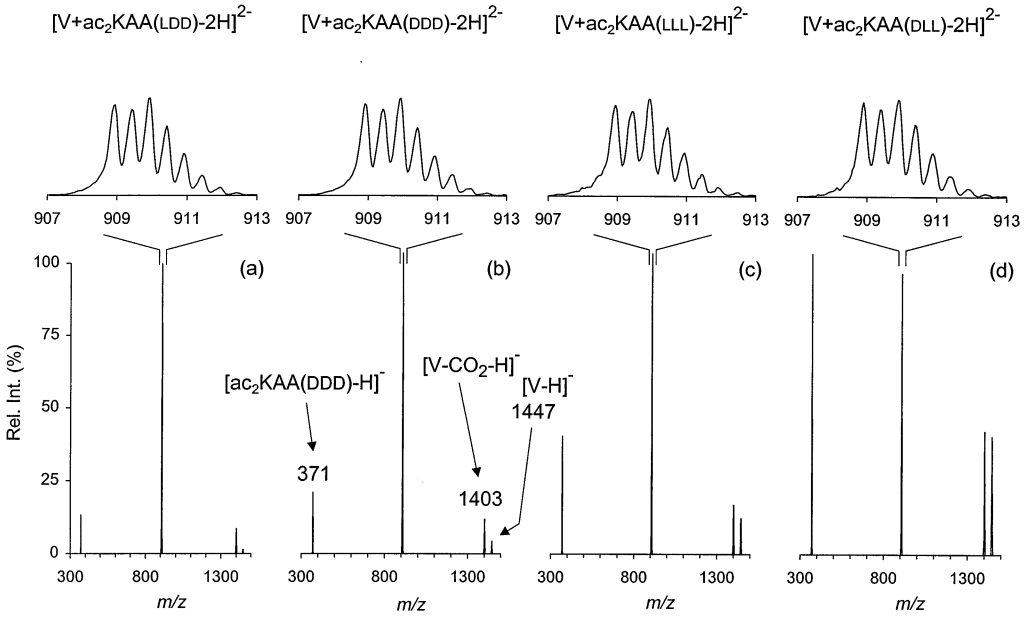


Fig. 2. Collision-induced dissociation (CID) spectra of dianionic noncovalent complexes formed between vancomycin (V) and tripeptide stereoisomers. The four isomeric complexes are (a) $[V + ac_2KAA(LDD)–2H]^{2-}$, (b) $[V + ac_2KAA(DDD)–2H]^{2-}$, (c) $[V + ac_2KAA(LLL)–2H]^{2-}$, (d) $[V + ac_2KAA(DLL)–2H]^{2-}$. The inset displays the isotope patterns of the precursor ions (m/z 908.9). The collision gas was argon at a pressure of 0.52 mTorr, collision energy was 70 eV.

Fig. 2(a)–(d) shows the negative ion CID mass spectra of the four isomeric $[V + ac_2KAA–2H]^{2-}$ complexes acquired at constant collision energy and constant collision gas pressure. The abundance of the fragment ions depends on the stereochemistry of the tripeptide and increases in the order: $ac_2KAA(LDD) < ac_2KAA(DDD) < ac_2KAA(LLL) < ac_2KAA(DLL)$. This indicates that the anionic complexes containing a –L-Ala residue are more readily dissociated than the complexes containing a –D-Ala residue. The complex in which the tripeptide has the same stereochemistry as the naturally occurring cell-wall precursor (i.e. LDD) gives the lowest abundance and the complex with the enantiomeric peptide (i.e. DLL) the highest abundance of fragments.

In contrast to the negatively charged complexes [Fig. 3(a)], the corresponding positively charged complexes formed between vancomycin and the tripeptide ligands did not display any significant differences in their gas phase stability [Fig. 3(b)].

Dissociation efficiency curves were generated to further investigate the relative energy of fragmentation of the isomeric $[V + ac_2KAA–2H]^{2-}$ complexes. Dissociation efficiency curves are plots of percent dissociation versus center-of-mass collision energy. Percent dissociation is calculated as the abundance of the tripeptide ion in proportion to the sum of the abundances of the noncovalent complex ion and the tripeptide ion. The relative positions of the dissociation curves are given by their intersection point energies (E_i), which can be used as a measure for the ease of dissociation of the noncovalent complex. The intersection point energy (E_i) corresponds to an apparent threshold energy for dissociation of the noncovalent complexes and E_i is determined as the intercept between the linear portion of the dissociation curve and the center-of-mass energy axis.

Table 1 lists the intersection energies for complexes formed between vancomycin or aglycovancomycin with the four stereoisomeric tripeptide ligands. The dissociation efficiency curves for the vancomycin–tripeptide complexes are shown in Fig. 4. The increase of percent dissociation with center-of-mass

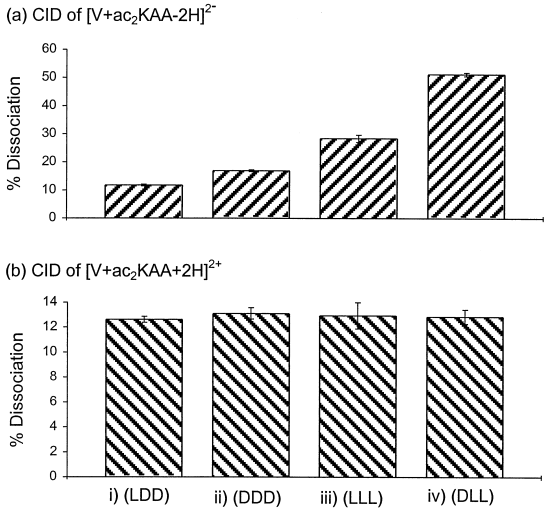


Fig. 3. Relative gas phase kinetic stabilities determined by CID of (a) dianionic and (b) dicationic noncovalent complexes formed between vancomycin (V) and tripeptide stereoisomers: (i) ac_2KAA(LDD), (ii) ac_2KAA(DDD), (iii) ac_2KAA(LLL), (iv) ac_2KAA(DLL). Percent dissociation is calculated as the relative intensity of the tripeptide ion in proportion to the sum of the intensities of the precursor ion and tripeptide ion. The ions were collided with argon at 70 eV collision energy, the collision gas pressure was 0.52 mTorr. The error bars show ± standard deviation of nine consecutive measurements.

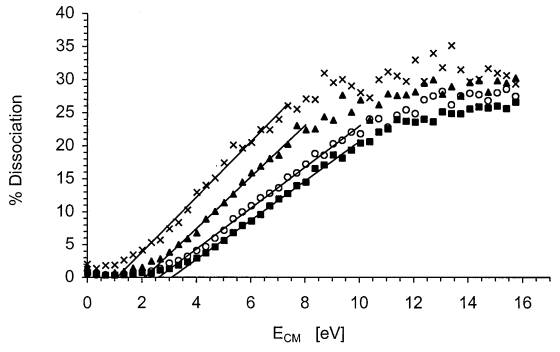


Fig. 4. Dissociation efficiency curves of four noncovalent vancomycin–tripeptide complexes: [vancomycin + tripeptide–2H]$^{2-}$. The four isomeric complexes are: (closed square) [V + ac_2KAA(LDD)–2H]$^{2-}$, (open circle) [V + ac_2KAA(DDD)–2H]$^{2-}$, (closed triangle) [V + ac_2KAA(LLL)–2H]$^{2-}$, (cross) [V + ac_2KAA(DLL)–2H]$^{2-}$. The ions were collided with xenon at single collision conditions, pressure 0.080 mTorr at various collision energies. The *y* axis represents percent dissociation, which is the relative intensity of the tripeptide ion (*m*/*z* 371) in proportion to the sum of the intensities of the precursor ion (*m*/*z* 908.9) and tripeptide ion.

collision energy is typical of that observed in low-energy collisional dissociation. There is a threshold energy and above this an almost linear rise in yield to an energy when the yield saturates and then remains approximately constant. Equivalent measurements made using argon as the collision gas were in agreement with Fig. 4 when expressed in terms of the centre-of-mass collision energy (data not shown).

As expected the intersection energies follow the same order as the relative abundances of the product ions in the CID spectra shown in Fig. 2(a)–(d). The order of gas phase stability for the negatively charged vancomycin–peptide complexes, as determined from their intersection energies, is: ac_2KAA(LDD) > ac_2KAA(DDD) > ac_2KAA(LLL) > ac_2KAA(DLL). This order is similar to that determined for the aglycovancomycin–tripeptide complexes.

Table 1
Intersection energies for dianionic noncovalent antibiotic–tripeptide ligand complexes[a]

	Antibiotics	
Tripeptide ligand	Vancomycin	Aglycovancomycin
ac_2KAA(LDD)	3.1	2.5
ac_2KAA(DDD)	2.6	2.4
ac_2KAA(LLL)	2.1	1.7
ac_2KAA(DLL)	1.1	0.9

[a] The intersection energy [E_i (eV)] is the center-of-mass collision energy at the intercept of the linear portion of the dissociation efficiency curve.

3.2. Order of solution association constants of four isomeric V + ac_2KAA complexes

To determine the order of solution association constants for the complexes formed between vancomycin and each of the tripeptide ligands we performed competitive binding experiments with two ligands and one receptor. The total receptor concentration was equal to the total ligand concentration.

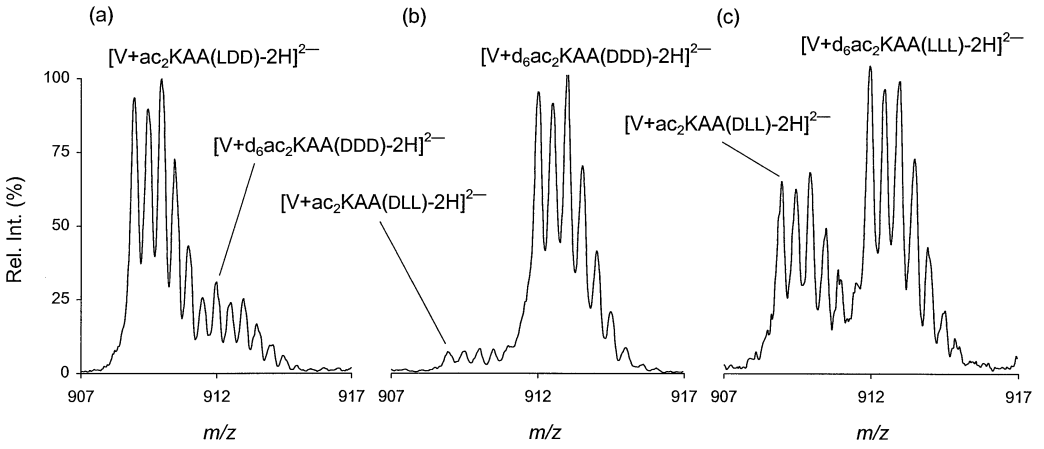


Fig. 5. Negative ion ESI mass spectra obtained from equimolar mixtures (50 μM, pH 5.1, 5 mM ammonium acetate) of vancomycin (V) and two tripeptide stereoisomers, one isotopically labeled. Displayed is the region of the mass spectrum, which shows the dianionic noncovalent complexes formed between vancomycin and the tripeptide stereoisomers. The equimolar mixtures were (a) V, ac_2KAA(LDD), and d_6ac_2KAA(DDD); (b) V, ac_2KAA(DLL), and d_6ac_2KAA(DDD); (c) V, ac_2KAA(DLL), and d_6ac_2KAA(LLL).

Using a deuterium labeled tripeptide and a nonlabeled tripeptide stereoisomer it was possible to determine the relative binding affinities of these ligands to vancomycin from the relative abundances of the complex ions observed in the ESI mass spectra. Fig. 5(a)–(c) shows the negative ion ESI mass spectra of three equimolar mixtures of vancomycin and two stereoisomeric peptide ligands (50 μM, 5 mM ammonium acetate, pH 5.1). From these spectra it is evident that ac_2KAA(LDD) has the highest solution association constant, followed by d_6ac_2KAA(DDD). The solution association constant for ac_2KAA(LDD) is 1.5×10^6 M^{-1} [16]. The two –L-Ala–L-Ala peptide ligands display a considerably reduced binding affinity compared to the –D-Ala–D-Ala ligands. The order of solution association constants is, as determined from negatively charged complex ion abundances: ac_2KAA(LDD) > d_6ac_2KAA(DDD) ≫ ac_2KAA(LLL) > d_6ac_2KAA(DLL). This order is similar to that determined for the gas phase stability of these complexes.

To verify that the deuterium labeling did not affect complex formation, a competition experiment was performed with equimolar amounts of vancomycin, ac_2KAA(DDD) and d_6ac_2KAA(DDD) (50 μM, 5 mM ammonium acetate buffer, pH 5.1). No difference in the relative abundances of the deuterated and nondeuterated complex ions were observed and the dissociation efficiency for the two complexes $[V + ac_2KAA(DDD)–2H]^{2-}$ and $[V + d_6ac_2KAA(DDD)–2H]^{2-}$ was also found identical (data not shown).

3.3. CID of mixed cluster ions comprised of an antibiotic and two peptide ligands

The decomposition of doubly charged mixed cluster ions with the general formulas $[A + L1 + L2–2H]^{2-}$ and $[A + L1 + L2 + 2H]^{2+}$, where A is the antibiotic, L1 and L2 represent the two peptide ligands, were then studied. These mixed clusters ions were observed in the ESI mass spectra of mixtures of an antibiotic and stereoisomeric peptide ligands at high ligand concentrations (>50 μM). Two types of fragmentation pathways for these mixed cluster ions can be observed: 1) charge separation by loss of a singly charged peptide ligand 2) neutral loss of a peptide ligand. In each reaction type loss of both ligands can occur, consequently there can be four competing fragmentation pathways in the decomposition of mixed cluster ions.

For mixed cluster ions comprised of an antibiotic,

a –D-Ala ligand, a –L-Ala ligand the relative abundance of the product ion consisting of a complex between an antibiotic and a –D-Ala peptide ligand is denoted I_{A+L1}; the relative abundance of the product ion consisting of a complex between an antibiotic and a –L-Ala peptide ligand is denoted I_{A+L2}. The branching ratio (I_{A+L1}/I_{A+L2}) for the charge separation reaction and the neutral loss reaction for positively charged mixed cluster ions is then calculated as the peak intensity ratios: $[A + L1 + H]^+/[A + L2 + H]^+$ and $[A + L1 + 2H]^{2+}/[A + L2 + 2H]^{2+}$, respectively. The branching ratios for the negatively charged mixed cluster ions is calculated by the corresponding ratios of negatively charged complex ion abundances. The branching ratios for the charge separation reactions of the mixed cluster ions comprised of two dipeptide ligands and an antibiotic were determined by the ratio of the dipeptide product ion abundances. For example, the I_{A+L1}/I_{A+L2} value for the charge separation reaction of [V + $d_3$acGA(L) + acGA(D)–2H]$^{2-}$ was determined by the product ion ratio of [$d_3$acGA(L)–H]$^-$ to [acGA(D)–H]$^-$, since the determination of the branching ratio by the product ion ratio of [V + acGA(D)–H]$^-$ to [V + $d_3$acGA(L)–H]$^-$ was complicated by their overlapping isotope distributions and the limited resolution of the mass-analyzing final quadrupole (Q3).

The branching ratio (I_{A+L1}/I_{A+L2}) for each reaction type (i.e. charge separation and neutral loss) is expected to become a measure of chiral recognition in the noncovalent mixed cluster ions. Thus, a branching ratio (I_{A+L1}/I_{A+L2}) > 1 means that the antibiotic binds more strongly a –D-Ala peptide enantiomer of a given peptide ligand (a –D-Ala peptide preference). The larger the I_{A+L1}/I_{A+L2} value from unity, the higher the degree of chiral recognition of the antibiotic. In contrast, $I_{A+L1}/I_{A+L2} < 1$, means that the antibiotic binds more strongly a –L-Ala peptide enantiomer of a given peptide ligand (a –L-Ala peptide preference). If $I_{A+L1}/I_{A+L2} = 1.0 \pm 0.1$ then the antibiotic cannot differentiate the chirality of a given peptide ligand.

The observed isotope pattern of the mixed cluster ion comprised of an antibiotic, and two stereoisomeric

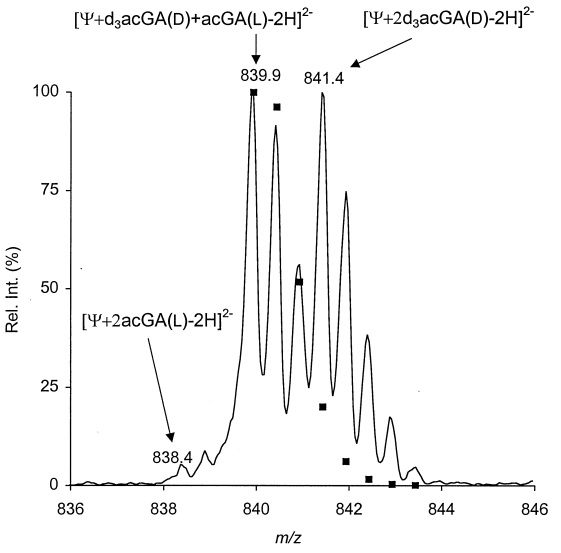


Fig. 6. Negative ion ESI mass spectrum obtained from a mixture of the pseudoaglycone of ristocetin (Ψ) and the peptide ligands $d_3$acGA(D), acGA(L). Displayed is the region with the three overlapping isotope patterns of the dianionic cluster ions. The filled squares show the theoretical isotope distribution for the mixed cluster ion comprised of the pseudoaglycone, $d_3$acGA(D), and acGA(L).

dipeptide ligands, such as [Ψ + $d_3$acGA(D) + acGA(L)–2H]$^{2-}$, inevitably contains a contribution from the (M + 3), (M + 4), (M + 5) natural abundant isotopes from the ion [Ψ + acGA(L) + acGA(L)–2H]$^{2-}$ (Fig. 6). The relative abundance of this nonlabeled dipeptide cluster ion was approximately 5% of the relative abundance of the mixed cluster ion [Ψ + $d_3$acGA(D) + acGA(L)–2H]$^{2-}$ (similar values were observed for the other mixed cluster ions, data not shown). The theoretical relative abundance of the (M + 3) isotope of the ion [Ψ + acGA(L) + acGA(L)–2H]$^{2-}$ is 20.1%. This means that the intensity of the monoisotopic peak of the mixed cluster ion [Ψ + $d_3$acGA(D) + acGA(L)–2H]$^{2-}$ contains at most 1% (0.05×0.201) of the ion [Ψ + acGA(L) + acGA(L)–2H]$^{2-}$. We have not made any corrections of the product ion intensities for this insignificant contribution, as it will not alter the stereoselective trends observed. For the mixed cluster ions comprised of an antibiotic, an isotopically la-

beled tripeptide and an unlabeled tripeptide stereoisomer, the monoisotopic peaks from the overlapping isotope distributions of the ions [antibiotic + ac_2KAA + ac_2KAA–2H]$^{2-}$ and [antibiotic + d_6ac_2KAA + ac_2KAA–2H]$^{2-}$ is separated by 3 Thomson. Consequently, the monoisopic peak of [antibiotic + d_6ac_2KAA + ac_2KAA–2H]$^{2-}$ contains a contribution from the (M + 6) natural abundant isotope(s) of [antibiotic + ac_2KAA + ac_2KAA–2H]$^{2-}$. In the case of pseudoaglycoristocetin–tripeptide mixed cluster ions the theoretical relative abundance of (M + 6) isotopes is less than 0.5%, which is a negligible contribution.

3.4. Vancomycin, aglycovancomycin, aglycovancomycinmethylester mixed cluster ions

Two charge separation and two neutral loss fragmentation pathways are observed upon CID of the positively charged mixed cluster ion [d_6ac_2KAA(LLL) + V + ac_2KAA(LDD) + 2H]$^{2+}$. The branching ratios and the four competing dissociation pathways [reactions (3)–(6)] for this ion are given as follows.

Charge separation:

$$\rightarrow [V + d_6ac_2KAA(LLL)+H]^+ + [ac_2KAA(LDD) + H]^+ \quad (3)$$

$$I_{A+L1}/I_{A+L2} = 1.08 \pm 0.02$$

$$\rightarrow [V + ac_2KAA(LDD) + H]^+ + [d_6ac_2KAA(LLL) + H]^+ \quad (4)$$

Neutral loss:

$$\rightarrow [V + d_6ac_2KAA(LLL) + 2H]^{2+} + ac_2KAA(LDD) \quad (5)$$

$$I_{A+L1}/I_{A+L2} = 1.06 \pm 0.05$$

$$\rightarrow [V + ac_2KAA(LDD) + 2H]^{2+} + d_6ac_2KAA(LLL) \quad (6)$$

The branching ratios for the competitive fragmentation reactions (3)–(6) are close to one. Thus, no stereoselective fragmentation of this positively charged mixed cluster ion is observed. In contrast, the corresponding negatively charged mixed cluster ion fragments stereoselectively by a preferential loss of [d_6ac_2KAA(LLL)–H]$^-$ [reaction (8)]. The same stereoselectivity occurs in the neutral loss reaction (10), which is the only neutral loss reaction observed—loss of neutral ac_2KAA(LDD) [reaction (9)] does not occur. The I_{A+L1}/I_{A+L2} values for the competing dissociation pathways for [V + d_6ac_2KAA(LLL) + ac_2KAA(LDD)–2H]$^{2-}$ are given as follows.

Charge separation:

$$\rightarrow [V + d_6ac_2KAA(LLL)–H]^- + [ac_2KAA(LDD)–H]^- \quad (7)$$

$$I_{A+L1}/I_{A+L2} = 2.62 \pm 0.05$$

$$\rightarrow [V + ac_2KAA(LDD)–H]^- + [d_6ac_2KAA(LLL)–H]^- \quad (8)$$

Neutral loss:

$$\nrightarrow [V + d_6ac_2KAA(LLL)–2H]^{2-} + ac_2KAA(LDD) \quad (9)^*$$

$$I_{A+L1}/I_{A+L2} > 100$$

$$\rightarrow [V + ac_2KAA(LDD)–2H]^{2-} + d_6ac_2KAA(LLL) \quad (10)$$

The CID mass spectrum (Fig. 7) of [d_6ac_2KAA(LLL) + V + ac_2KAA(LDD)–2H]$^{2-}$ (m/z 1099.1) shows the relative abundance of the five product ions from the three competing dissociation pathways [reactions (7), (8), and (10)] and with lower abundance singly charged decarboxylated vancomycin [V–H–CO_2]$^-$ (m/z 1403). The high degree of stereoselective dissociation for the neutral loss reaction is noted by the absence of a peak at m/z 913, which would correspond to [V + d_6ac_2KAA(LLL)–2H]$^{2-}$.

The branching ratios for the charge separation dissociation pathways of negatively charged vancomycin and aglycovancomycin mixed cluster ions are listed in Table 2. The I_{A+L1}/I_{A+L2} values are between 2.5 and 22 for mixed cluster ions containing ac_2KAA(XDD) and d_6ac_2KAA(XLL) (X denotes either L

* Reaction (9) not observed.

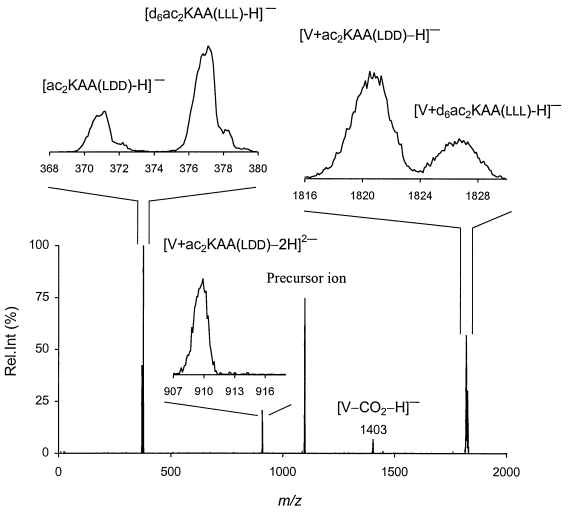


Fig. 7. Negative ion CID spectrum of the dianionic mixed cluster ion comprised of vancomycin (V), ac_2KAA(LDD), and the deuterium labeled stereoisomer d_6ac_2KAA(LLL).

or D configuration). This means there is a preferential stereoselective loss of anionic d_6ac_2KAA(XLL) ligands. The neutral loss reactions occur with an even higher degree of stereoselective dissociation, since their I_{A+L1}/I_{A+L2} values are larger than 100. The mixed cluster ions containing a set of dipeptide enantiomers exhibit a lower degree of chiral discrimination, as their I_{A+L1}/I_{A+L2} values are between 1.2 and 2.0. The branching ratios of the neutral loss reactions for the dipeptide mixed cluster ions are difficult to determine with a triple quadrupole instrument. This is due to the low abundance of the doubly charged product ions combined with the need for a high resolution in Q3 in order to resolve the isotopomers. However, the peakwidths (at half peak height) obtained from the CID spectra acquired at low Q3 mass resolution allow an estimate of the stereoselectivity of the neutral loss reactions. CID of [V + acAA(DD) + $d_3$acAA(LL)–2H]$^{2-}$ gives a composite peak consisting of [V + acAA(DD)–2H]$^{2-}$ and [V + $d_3$acAA(LL)–2H]$^{2-}$, which results from neutral loss of $d_3$acAA(LL) and acAA(DD), respectively. The width of this composite peak is approximately 40% wider than that of [V + acAA(DD)–2H]$^{2-}$ (obtained from CID of [V + 2acAA(DD)–2H]$^{2-}$) (data not shown). If the neutral loss reactions of [V + acAA(DD) + $d_3$acAA(LL)–2H]$^{2-}$ occurred with high stereoselectivity ($I_{A+L1}/I_{A+L2} > 100$), then the two peak widths would be very similar. In case of no stereoselectivity ($I_{A+L1}/I_{A+L2} = 1$), the width of the composite peak would be theoretically 40%–45% larger than that of [V + acAA(DD)–2H]$^{2-}$. Therefore, the neutral loss reactions for this dipeptide mixed cluster ion occur with a I_{A+L1}/I_{A+L2} value close to one. Similar results were obtained for the dipeptide mixed cluster ions [V + acGA(D) + $d_3$acGA(L)–2H]$^{2-}$ and [V + acAG(D) + $d_3$acAG(L)–2H]$^{2-}$ (data not shown). This fragmentation pattern is in marked contrast to the very high degree of stereoselectivity for the neutral loss reactions which are observed for the dianionic tripeptide mixed cluster ions (Table 2).

Unlike the above-mentioned cluster ions the aglycovancomycinmethylester (a$VOCH_3$) mixed cluster ions do not fragment by neutral loss of peptide ligand. The only fragmentation pathways observed for the ions [a$VOCH_3$ + d_6ac_2KAA(LLL) + ac_2KAA(LDD)–2H]$^{2-}$ and [a$VOCH_3$ + $d_3$acAA(LL) + acAA(DD)–2H]$^{2-}$ are charge separation reactions, i.e. loss of anionic peptide ligands. The I_{A+L1}/I_{A+L2} values for their charge separation reactions are 27 and 2.0, respectively. The highest degree of stereoselectivity ($I_{A+L1}/I_{A+L2} = 27$) is thus observed for the mixed cluster ion containing the tripeptides.

The decomposition of mixed cluster ions with the composition [acAA + V + ac_2KAA–2H]$^{2-}$ was also investigated. For these dipeptide–tripeptide–vancomycin cluster ions only one or two competitive fragmentation pathways are observed:

$$[\text{acAA(DD)} + \text{V} + \text{ac}_2\text{KAA(LDD)}–2\text{H}]^{2-} \nearrow [\text{V} + \text{acAA(DD)}–\text{H}]^- + [\text{ac}_2\text{KAA(LDD)}–\text{H}]^- \quad (11)$$

$$\searrow [\text{V} + \text{ac}_2\text{KAA(LDD)}–2\text{H}]^{2-} + \text{acAA(DD)} \quad (12)$$

$$[\mathrm{acAA(LL)} + \mathrm{V} + \mathrm{ac_2KAA(LDD)}\text{–}2\mathrm{H}]^{2-} \nearrow [\mathrm{V} + \mathrm{acAA(LL)}\text{–}\mathrm{H}]^{-} + [\mathrm{ac_2KAA(LDD)}\text{–}\mathrm{H}]^{-} \quad (13)$$

$$\searrow [\mathrm{V} + \mathrm{ac_2KAA(LDD)}\text{–}2\mathrm{H}]^{2-} + \mathrm{acAA(LL)} \quad (14)$$

$$[\mathrm{acAA(DD)} + \mathrm{V} + \mathrm{ac_2KAA(LLL)}\text{–}2\mathrm{H}]^{2-} \nearrow [\mathrm{V} + \mathrm{acAA(DD)}\text{–}\mathrm{H}]^{-} + [\mathrm{ac_2KAA(LLL)}\text{–}\mathrm{H}]^{-} \quad (15)$$

$$\not\searrow [\mathrm{V} + \mathrm{ac_2KAA(LLL)}\text{–}2\mathrm{H}]^{2-} + \mathrm{acAA(DD)} \quad (16)^{*}$$

All the charge separation reactions yield $[\mathrm{ac_2KAA}\text{–}\mathrm{H}]^{-}$ and $[\mathrm{V} + \mathrm{acAA}\text{–}\mathrm{H}]^{-}$. Thus, there is only one charge separation pathway of each mixed cluster ion, namely loss of anionic tripeptide [reactions (11), (13), and (15)]. The stereochemical configuration of the dipeptide ligands does not affect this selective loss of tripeptide ligands. Clearly other factors than chirality are determining the selective loss of $[\mathrm{ac_2KAA(LXX)}\text{–}\mathrm{H}]^{-}$ in the charge separation reactions. In the neutral loss reactions, however, an intriguing difference between tripeptide stereoisomers can be observed. The only neutral loss observed is the dipeptide, but the reaction only occurs when $\mathrm{ac_2KAA(LDD)}$ is present [reactions (12) and (14)]. From these fragmentation patterns it is seen that the charge separation reactions occur without any stereoselectivity, whereas the neutral loss reactions are clearly dependent upon the chirality of the tripeptide.

3.5. Pseudoaglycoristocetin (Ψ) mixed cluster ions

All dianionic pseudoaglycoristocetin mixed cluster ions containing a –D-Ala– ligand and a –L-Ala– ligand fragment stereoselectively with a preferential loss of a –L-Ala ligand. That is, CID yields predominantly product ions in which the antibiotic is associated with a –D-Ala ligand. The degree of stereoselectivity in the charge separation reactions is significantly higher than that observed for the corresponding vancomycin and aglycovancomycin mixed cluster ions. In particular, a remarkable high degree of stereoselectivity is observed in the dissociation of the ions comprised of pseudoaglycoristocetin, $\mathrm{ac_2KAA(XDD)}$ and $\mathrm{d_6ac_2KAA(XLL)}$ (X denotes either L or D configuration). The branching ratio for these ions is above 55 which means that more than 98% of the ions dissociate by a stereoselective loss of the assumed nonspecifically bound $\mathrm{d_6ac_2KAA(XLL)}$. The branching ratios for the fragmentation pathways of negatively charged Ψ mixed cluster ions are listed in Table 3. The CID spectrum (Fig. 8) of $[\mathrm{d_6ac_2KAA(LLL)} + \Psi + \mathrm{ac_2KAA(LDD)}\text{–}2\mathrm{H}]^{2-}$ (m/z 1026.1) shows that the predominant fragmentation pathway is a stereoselective charge separation reaction, with loss of $[\mathrm{d_6ac_2KAA(LLL)}\text{–}\mathrm{H}]^{-}$ (m/z 376.9) [reaction (17)]. None of the negatively charged Ψ-mixed cluster ions fragment by neutral loss of peptide ligands

$$[\mathrm{d_6ac_2KAA(LLL)} + \Psi + \mathrm{ac_2KAA(LDD)}\text{–}2\mathrm{H}]^{2-} \rightarrow [\Psi + \mathrm{ac_2KAA(LDD)}\text{–}\mathrm{H}]^{-} + [\mathrm{d_6ac_2KAA(LLL)}\text{–}\mathrm{H}]^{-} \quad (17)$$

Most of the positively charged Ψ mixed cluster ions do not reveal any significant stereoselective reactions upon CID. The positively charged mixed

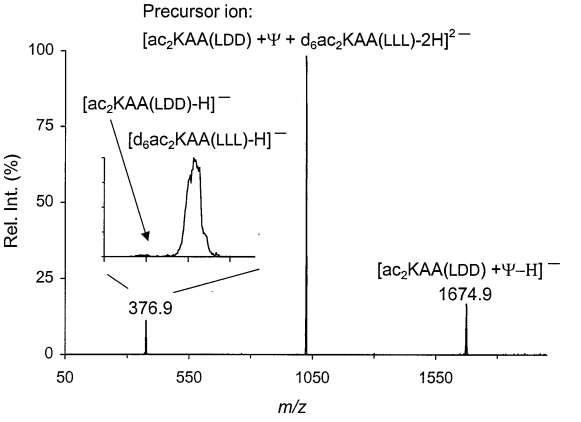


Fig. 8. Negative ion CID spectrum of the dianionic mixed cluster ion comprised of the pseudoaglycone of ristocetin (Ψ), $\mathrm{ac_2KAA(LDD)}$, and the deuterium labeled stereoisomer $\mathrm{d_6ac_2KAA(LLL)}$.

* Reaction (16) not observed.

Table 2
The branching ratios (I_{A+L1}/I_{A+L2}) for the charge separation dissociation pathways of dianionic vancomycin (V) and aglycovancomycin (aV) mixed cluster ions with the general formula $[A + L1 + L2–2H]^{2-}$, where A is the antibiotic, L1 and L2 represent the two peptide ligands; the branching ratios for the neutral loss dissociation pathways for the ions, where L1 and L2 are tripeptides, were all above 100

L1	L2	I_{A+L1}/I_{A+L2}		Relationship between L1 and L2
		V	aV	
acGA(D)	$d_3$acGA(L)	1.4	1.3	Enantiomers
acAG(D)	$d_3$acAG(L)	1.3	1.4	Enantiomers
acAA(DD)	$d_3$acAA(LL)	1.6	1.9	Enantiomers
ac_2KAA(LDD)	d_6ac_2KAA(LLL)	2.6	5.0	Diastereomers
d_6ac_2KAA(DDD)	ac_2KAA(DLL)	15.4	21.5	Diastereomers
ac_2KAA(DDD)	d_6ac_2KAA(LLL)	6.7	6.5	Enantiomers
ac_2KAA(LDD)	d_6ac_2KAA(DLL)	9.8	12.6	Enantiomers
ac_2KAA(LDD)	d_6ac_2KAA(DDD)	1.5	1.7	Diastereomers, both D-Ala's

cluster ions, which contain a –D-Ala– ligand and a –L-Ala– ligand, show in general no significant preferential loss of the –L-Ala– ligand in the charge separation reactions, i.e. their I_{A+L1}/I_{A+L2} values are very close to 1.0. The notable exceptions are the ions: $[ac_2KAA(LDD) + \Psi + d_6ac_2KAA(DLL) + 2H]^{2+}$ ($I_{A+L1}/I_{A+L2} = 1.9$) and $[ac_2KAA(DDD) + \Psi + d_6ac_2KAA(LLL) + 2H]^{2+}$ ($I_{A+L1}/I_{A+L2} = 0.6$). The former ion fragments by a preferential loss of protonated $d_6ac_2KAA(DLL)$, whereas the latter ion predominantly loses protonated $ac_2KAA(DDD)$. No neutral loss of tripeptide ligand was observed for the positively charged mixed cluster ions comprised of Ψ, $ac_2KAA(XDD)$ and $d_6ac_2KAA(XLL)$ (X denotes either L or D configuration). In contrast, dissociation of the positively charged dipeptide-Ψ mixed cluster ions occurs predominantly by neutral loss of dipeptide and to a lesser degree by charge separation. However, dissociation by the charge separation reactions is favoured relative to the neutral loss reactions for $[d_3acAAA(DDD) + \Psi + acAAA(LLL) + 2H]^{2+}$. The neutral loss reactions for the positively charged Ψ mixed cluster ions occur with a very low degree of stereoselectivity, i.e. their I_{A+L1}/I_{A+L2} values are very close to 1.0 (data not shown). The procedure for estimation of the degree of stereoselectivity in the neutral loss reactions for the positively charged Ψ mixed cluster ion is identical to that described for the vancomycin mixed cluster ions Sec. 3.4. The branching ratios for the charge separation dissociation pathways of positively charged Ψ mixed cluster ions are listed in Table 3.

3.6. Ristocetin mixed cluster ions

Unexpectedly, the negatively charged ristocetin mixed cluster ions show a completely different stereoselective fragmentation behavior than that of pseudoaglycoristocetin mixed cluster ions. To illustrate this contrasting behavior the fragmentation pathways for $[d_6ac_2KAA(LLL) + R + ac_2KAA(LDD)–2H]^{2-}$ are shown as follows.

Charge separation:

$$\rightarrow [R + d_6ac_2KAA(LLL)–H]^- + [ac_2KAA(LDD)–H]^- \quad (18)$$

$$I_{A+L1}/I_{A+L2} = 0.47 \pm 0.05$$

$$\rightarrow [R + ac_2KAA(LDD)–H]^- + [d_6ac_2KAA(LLL)–H]^- \quad (19)$$

As can be seen, this mixed cluster ion loses predominantly $[ac_2KAA(LDD)–H]^-$ [reaction (18)]. The same is true for $[d_6ac_2KAA(LLL) + R + ac_2KAA(DDD)–2H]^{2-}$ (see Table 4). Apparently, ristocetin has a stronger preference for binding to $d_6ac_2KAA(LLL)$ than to $ac_2KAA(LDD)$ or $ac_2KAA(DDD)$ in the gas phase. This is in marked

Table 3
The branching ratios (I_{A+L1}/I_{A+L2}) for the charge separation dissociation pathways of doubly positively charged (Pos.) and doubly negatively charged (Neg.) pseudoaglycoristocetin (Ψ) mixed cluster ions with the general formulas $[\Psi + L1 + L2–2H]^{2-}$ and $[\Psi + L1 + L2 + 2H]^{2+}$, where L1 and L2 represent the two peptide ligands

L1	L2	I_{A+L1}/I_{A+L2} Neg.	I_{A+L1}/I_{A+L2} Pos.	Relationship between L1 and L2
$d_3$acGA(D)	acGA(L)	24	1.0	Enantiomers
$d_3$acAG(D)	acAG(L)	6.0	1.1	Enantiomers
acAA(DD)	$d_3$acAA(LL)	9.0	1.0	Enantiomers
acAA(DD)	$d_3$acAG(D)	4.2	0.3	Both contain a –D-Ala residue
acGA(D)	acAA(DD)	2.5	3.5	Both contain a –D-Ala residue
$d_3$acGA(D)	acAG(D)	13	1.1	Both contain a –D-Ala residue
$d_3$acAG(D)	acGA(L)	4.9	···	
acAA(DD)	acGA(L)	11	0.3	
ac_2KAA(LDD)	d_6ac_2KAA(LLL)	>55	1.2	Diastereomers
d_6ac_2KAA(DDD)	ac_2KAA(DLL)	>55	1.1	Diastereomers
$d_3$acAAA(DDD)	acAAA(LLL)	13	1.1	Enantiomers
ac_2KAA(DDD)	d_6ac_2KAA(LLL)	>55	0.6	Enantiomers
ac_2KAA(LDD)	d_6ac_2KAA(DLL)	>55	1.9	Enantiomers
d_6ac_2KAA(DDD)	ac_2KAA(LDD)	0.8	0.6	Diastereomers, both D-Ala's

contrast to the high degree of stereoselective binding to ac_2KAA(LDD) or ac_2KAA(DDD), which is observed for the corresponding pseudoaglycoristocetin mixed cluster ions. However, the ristocetin mixed cluster ions $[d_6ac_2KAA(DDD) + R + ac_2KAA(DLL)–2H]^{2-}$ and $[d_6ac_2KAA(DLL) + R + ac_2KAA(LDD)–2H]^{2-}$ exhibit the same preference for binding to the ac_2KAA(XDD) peptides as the corresponding pseudoaglycoristocetin ions (although with a significant lower degree of stereoselectivity). The branching ratios for the investigated ristocetin mixed cluster ions are listed in Table 4.

Interestingly, the fragmentation behavior of the positively charged ristocetin–tripeptide mixed cluster ion closely resembles that of the corresponding pseudoaglycoristocetin ions. In particular, the preferential loss of d_6ac_2KAA(DLL) from $[ac_2KAA(LDD) + R + d_6ac_2KAA(DLL) + 2H]^{2+}$ and the preferential loss of ac_2KAA(DDD) from $[ac_2KAA(DDD) + R + d_6ac_2KAA(LLL) + 2H]^{2+}$ is similar to that of the corresponding pseudoaglycoristocetin ions. The positively charged ristocetin–dipeptide mixed cluster ion fragmented only by loss of neutral dipeptide. The neutral loss reactions for the positively charged ristocetin–dipeptide mixed cluster ions occur with a very low degree of stereoselectivity, i.e. their I_{A+L1}/I_{A+L2} values are very close to 1.0 (data not shown). The procedure for estimation of the degree of stereoselectivity in the neutral loss reactions for these ions is identical to that described for the vancomycin mixed cluster ions in the preceding section.

3.7. Order of proton affinities and gas phase acidities for the peptide ligands

In order to assess the importance of the gas phase acidity and proton affinity of the peptide ligands for the dissociation of the noncovalent complexes, we have determined the order of gas phase acidities and proton affinities for the peptide ligands. The gas phase acidity (ΔH_{ACID}) of a peptide ligand (L) is the enthalpy change for the reaction $LH \rightarrow L^- + H^+$, and it is equivalent to the proton affinity of the deprotonated peptide ligand (L^-). The proton affinity (PA) of L is the enthalpy change for the reaction $LH^+ \rightarrow L + H^+$. The order of gas phase acidities and proton affinities of the peptide ligands was estimated by the kinetic method, in which measurement of the relative fragment ion abundances (from the decomposition of proton bound peptide dimers) is used to determine differences in the gas phase acidities and proton affinities of the monomers [35–37].

Table 4
The branching ratios (I_{A+L1}/I_{A+L2}) for the charge separation dissociation pathways for doubly positively charged (Pos.) and doubly negatively charged (Neg.); ristocetin (R) mixed cluster ions with the general formulas $[R + L1 + L2–2H]^{2-}$ and $[R + L1 + L2 + 2H]^{2+}$, where L1 and L2 represent the two peptide ligands

L1	L2	I_{A+L1}/I_{A+L2} Neg.	I_{A+L1}/I_{A+L2} Pos.[a]	Relationship between L1 and L2
$d_3$acGA(D)	acGA(L)	1.2	···	Enantiomers
acAA(DD)	$d_3$acAA(LL)	2.5	nr	Enantiomers
acAA(DD)	acGA(D)	0.4	nr	Both contain a –D-Ala residue
acAA(DD)	acGA(L)	0.5	nr	
ac_2KAA(LDD)	d_6ac_2KAA(LLL)	0.5	1.4	Diastereomers
d_6ac_2KAA(DDD)	ac_2KAA(DLL)	18	1.0	Diastereomers
$d_3$acAAA(DDD)	acAAA(LLL)	1.2	1.3	Enantiomers
ac_2KAA(DDD)	d_6ac_2KAA(LLL)	0.6	0.6	Enantiomers
ac_2KAA(LDD)	d_6ac_2KAA(DLL)	6.0	1.7	Enantiomers
d_6ac_2KAA(DDD)	ac_2KAA(LDD)	2.0	0.5	Diastereomers, both D-Ala's

[a] nr = no reaction observed.

The order of gas phase acidities (ΔH_{ACID}) of the peptide ligands is (in decreasing order)

acAG > acAA > acGA > acAAA > ac_2KAA(LLL) = ac_2KAA(DDD) > ac_2KAA(LDD) = ac_2KAA(DLL)

The order of gas phase acidities of peptide stereoisomers was determined by decomposition of cluster ions comprised of a peptide and an isotopically labeled stereoisomer. Decomposition of cluster ions with the composition [dipeptide + ac_2KAA–H]$^-$ yields only [ac_2KAA–H]$^-$ and hence ΔH_{ACID}(dipeptide) $\gg$ ΔH_{ACID}(ac_2KAA). Decomposition of the cluster ion with the composition [ac_2KAA(LDD) + d_6ac_2KAA(LLL)–H]$^-$ yields primarily [ac_2KAA(LDD)–H]$^-$ and hence ΔH_{ACID} [d_6ac_2KAA(LLL)] > ΔH_{ACID}[ac_2KAA(LDD)]. Consistent with this order of gas phase acidities CID of [ac_2KAA(LDD) + d_6ac_2KAA(DDD)–H]$^-$ and [ac_2KAA(DDD) + d_6ac_2KAA (DLL)–H]$^-$ yields primarily [ac_2KAA(LDD)–H]$^-$ and [d_6ac_2KAA(DLL)–H]$^-$, respectively. The fragment ion ratio of [ac_2KAA(LDD)–H]$^-$ to [d_6ac_2KAA(LLL)–H]$^-$ was 8.5 (from the decomposition of [ac_2KAA(LDD) + d_6ac_2KAA (LLL)–H]$^-$) at the conditions listed in the experimental section. No other fragment ions than singly charged peptides could be observed in the CID spectra of the negatively charged cluster ions.

The order of gas phase proton affinities of the peptide ligands is (in decreasing order)

ac_2KAA(LLL) = ac_2KAA(DDD) > ac_2KAA(LDD) = ac_2KAA(DLL) > acAAA > acAA > acAG > acGA

4. Discussion

The most remarkable results of this study are the pronounced differences in gas phase stabilities of the vancomycin–tripeptide complexes observed between positive and negative ion mode reflecting loss or retainment of chiral specificity, respectively, and also that stereoselective decomposition of mixed cluster ions with a preferential binding to –D-Ala peptides is only observed in negative ion mode. However, unexpected exceptions were found for the ristocetin mixed cluster ions. Below these observations will be attempted to be explained based on considerations of the different properties of noncovalent complexes in the two very different environments: the gas phase and the solution phase.

The dissociation of a noncovalently bound complex in the gas phase differs in several important ways from the equivalent reaction in solution. In solution

the enthalpy of the reaction can be divided between an endothermic dissociation process, where the bonds between the acceptor and the ligand are broken, and an exothermic resolvation of the surfaces, which were involved in the formation of the complex. Entropically, the increase in the translational degrees of freedom favours the dissociation, but on the other hand, the disappearance of the vibrational degrees of freedom associated with the intermolecular bonds in the complex is entropically unfavourable. The relative importance of the enthalpic and entropic contributions to the dissociation constant may differ considerably depending on the acceptor and the ligand as expressed in enthalpic–entropic compensation relationships [38,39]. In the gas phase a very different situation prevails. Any dissociation without charge separation will be endothermic, but on the other hand, the electrostatic interactions between the acceptor and the ligand will be far more important than in solution and may determine whether a charge separation reaction is endo- or exothermic.

The differences between the reactions in solution and gas phase must be kept in mind when discussing the results described previously. The complex contributions from both entropic and enthalpic terms to the reaction free energy in solution mean that any correlation between the dissociation constant in solution and the stability in the gas phase may be fortuitous. However, when the changes in the stereochemistry of the ligand influences the stability of the complex in gas phase in the same direction as in solution, structural similarities of the complex in the two phases must be assumed. Thus the parallel between the reactivity of the dianionic vancomycin–tripeptide complexes and the association constant in solution indicate that the structures of the complex in the two phases are similar.

4.1. Relative gas phase stabilities of isomeric vancomycin–tripeptide complexes

In solution, vancomycin–peptide complexes have a zwitterionic charge distribution and a zero net charge at pH 5 [Fig. 1(a)]. The electrospray ionization process generates predominantly doubly positively or doubly negatively charged complexes in positive and negative ion mode, respectively. The addition or removal of protons in the ESI process of forming dicationic or dianionic complexes may interfere with the structurally specific interaction between antibiotic and peptide. In order to have a specific interaction between peptide and antibiotic, an anionic C-terminus of the peptide is required. Addition of protons to a gas phase complex can be likened to a decrease in pH. In solution, a decrease in pH to below 3.5 causes dissociation of the complex because of protonation of the anionic peptide ligand [22]. We assume that a somewhat similar situation exists in the positively charged complexes in which the C-terminus of the peptide ligand becomes neutral. In contrast, the charge sites in the negatively charged complexes are presumably the C-termini of the interaction partners. Consequently, negatively charged complexes have the essential requirement for specific interaction fulfilled.

The order of solution association constants is, as determined from the competition experiments: $ac_2KAA(\text{LDD}) > d_6ac_2KAA(\text{DDD}) \gg ac_2KAA(\text{LLL}) > d_6ac_2KAA(\text{DLL})$. This order is similar to that determined for the gas phase stability of the dianionic complexes. The implication then is, in the gas phase, the interaction between vancomycin and peptide ligands containing D-Ala residues is stronger than the corresponding interaction with peptide ligands containing L-Ala residues. The most stable complex in the gas phase is the complex formed between vancomycin and $ac_2KAA(\text{LDD})$. Substitution of the L-Lys by D-Lys reduces the gas phase stability. A similar preference for an L residue in position three of the peptide sequence is also observed in solution. From these experiments it is clear that the gas phase stabilities of the negatively charged vancomycin–tripeptide complexes are markedly influenced by the chirality of the three residues in the tripeptide ligand. The stereoselective fragmentation of dianionic mixed cluster ions and the order of apparent dissociation threshold energies of the four isomeric $[V + ac_2KAA–2H]^{2-}$ complexes strongly suggest that in anionic antibiotic–peptide complexes the structurally specific interaction between peptide and antibiotic is retained from solution into gas phase. The charge sites in the dianionic

vancomycin–tripeptide complexes is then believed to be the C-termini of the interaction partners. In such complexes the vancosamino group and the N-terminus of vancomycin are neutral. This is different from solution in which the amino groups are protonated (at pH 5). However, binding studies on N-acetyl and N,N′-diacetyl derivatives of vancomycin (N and N′ represents the N-terminus and vancosamino group, respectively) have shown that a charged N-terminus and vancosamino group are not required for complex formation, although acetylation of them leads to a reduction in binding energy [11,40]. Also, the pH dependence of both rates and equilibrium constants of the binding of ac_2KAA(LDD) to vancomycin suggest that a protonated N-terminus of vancomycin is not essential to strong binding [19].

In solution, the charge sites of the complex are solvated and between the charge sites there is water, which means that the Coulomb repulsion between equal charges will be reduced by the dielectric contant of water ($\epsilon \sim 80$). This screening effect is not present in the gas phase ($\epsilon = 1$), and thus Coulomb repulsion is increased upon transfer of a multiply charged complex from aqueous solution to the vacuum. The Coulomb repulsion energy in the dianionic vancomycin–ac_2KAA(LDD) complex is thus much higher in gas phase than it would be in solution. Despite the increased Coulomb repulsion energy it appears that the structurally specific interaction between vancomycin and tripeptide in dianionic complexes is not destroyed. It may, however, be perturbed relative to the solution structure, but with a retention of the structural features which are responsible for the stereoselective binding of –D-Ala peptides.

The order of gas phase stabilities for the dianionic vancomycin–ac_2KAA complexes is similar to that determined for the aglycovancomycin–ac_2KAA complexes, which implies that the sugar groups are not directly involved in the interaction between the peptide ligand and the antibiotic in the gas phase, and that we are indeed probing the intrinsic ligand affinities of the binding pocket. The observation that vancomycin–tripeptide complexes give higher intersection energies than the corresponding aglycovancomycin–tripeptide complexes is attributable to an increase in the number of degrees of freedom. The presence of the sugar moiety increases the number of vibrational and rotational modes over which the internal energy acquired by the collision process is to be distributed. Consequently, vancomycin–tripeptide complexes require a higher energy input to fragment at the same rate as the aglycovancomycin–tripeptide complexes.

The position of the dissociation curves is markedly influenced by the ESI conditions. A low voltage difference between the tube lens and the skimmer, i.e. mild declustering conditions, leads to a higher intersection energy. This shift is caused by a decrease in the internal energy of the ions, i.e. “cold” ions are generated in the ESI interface at mild declustering conditions. A similar effect on the dissociation efficiency can be observed by changing the temperature of the heated capillary in the ESI interface. Alteration of the ESI conditions, however, did not influence the order of gas phase stability of the isomeric $[V + ac_2KAA–2H]^{2-}$ complexes.

The activation barrier for the dissociation of $[V + ac_2KAA–2H]^{2-}$ is sufficiently high for subsequent covalent bond cleavage (loss of carbon dioxide) to occur in the vancomycin product ion. The CID spectra of the four stereoisomeric $[V + ac_2KAA–2H]^{2-}$ complexes [Fig. 2(a)–(d)] show that the peak height ratio of the decarboxylated vancomycin ion $[V–CO_2–H]^-$ (m/z 1403) to the vancomycin ion $[V–H]^-$ (m/z 1447) increases with the intersection energy of the complexes, i.e. a high intersection energy results in extensive fragmentation of vancomycin as shown by the loss of carbon dioxide. A high intersection energy corresponds to a high activation barrier for dissociation. Surpassing such a barrier results in the formation of vancomycin ions with a higher internal energy than vancomycin ions formed by dissociation of complexes with a lower activation barrier. Consequently, the rate for subsequent fragmentation (loss of carbon dioxide) is highest for the “hottest” vancomycin ions. Upon CID of negatively charged vancomycin, decarboxylation is the preferred fragmentation pathway (the lowest threshold energy) and as such no other product ions could be observed in the CID spectra obtained at low collision energies. The occurrence of covalent bond cleavage upon dissociation of nonco-

valent complexes has also been observed for polyether–ammonium ion complexes [41] and aldose reductase–$NADP^+$ complexes [42].

In contrast to the negatively charged complexes the positively charged complexes do not show any significant differences in their reactivity. This might be surprising, however, if a dicationic gas phase vancomycin–peptide complex retains its zwitterionic charge distribution from solution then two protons should be added to a complex having two ammonium cations and two carboxylate anions. This leads to a complex having a zwitterionic charge distribution with four positively charged sites and two negatively charged sites. Formation of a zwitterion in the gas phase, however, is normally energetically unfavourable without solvent stabilization [43,44]. It is therefore highly likely that the similarity in the reactivity of isomeric positively charged complexes is due to protonation of the anionic C-terminus of the ligand. This leads to a weakening of the hydrogen bonds normally formed between the anionic C-terminus of the ligand and the amide hydrogens in the antibiotic peptide backbone. Retention of the ligand in the binding pocket is therefore much less favourable.

It should be noted that singly protonated bradykinin is expected to have a gas phase zwitterionic charge distribution in which the N-terminal and C-terminal guanidino groups of the arginine residues are positively charged and form salt bridges to the negatively charged C-terminus [45]. Such a structure is only energetically feasible when highly basic proton acceptors, such as arginine, are in close proximity with good proton donors, such as carboxylic and sulfonic acid groups (the sidechain in cysteic acid) [46,47]. Therefore, it is reasonable to assume that no structural specific interaction exists in the positively charged antibiotic-peptide complexes since a structurally specific interaction in such a complex requires a zwitterionic charge distribution where the opposite charges are not forming salt bridges. For example in the vancomycin–ac_2KAA(LDD) complex (in solution) the amino group of vancosamine points away from the binding cleft. Consequently, the charged group is available for maximum solvation but is by no means optimised for direct electrostatic interaction with the peptide carboxylate anion (intercharge distance 15 Å). The same is true in the case of the protonated N-terminus of vancomycin and the peptide carboxylate anion (intercharge distance 5 Å) [11]. Thus, the formation of salt bridges in gas phase is not possible without disrupting the structurally specific interaction between antibiotic and peptide. Further, the proton affinities of the amino groups in vancomycin are substantially smaller than that of the guanidino group of arginine. For this reason salt bridge formation should not be expected to occur in cationic antibiotic–peptide complexes. We assume that the carboxylic groups in the antibiotic–peptide complexes are neutral and that the two ionizing protons in the doubly protonated antibiotics and their complexes are located at the two amino groups, since they have the highest proton affinity and it ensures a large intercharge distance. Note that protons are not necessarily located at the most basis sites if the alternative of greater intercharge distance is available [48].

4.2. Pseudoaglycoristocetin (Ψ) mixed cluster ions

The charge separation reactions for the dianionic mixed cluster ions containing ac_2KAA(XDD), d_6ac_2KAA(XLL), and Ψ occur with a very high degree of stereoselectivity ($I_{A+L1}/I_{A+L2} > 55$). The preferential loss of anionic d_6ac_2KAA(XLL) indicates that the binding energy between this ligand and the antibiotic is significantly lower than the binding energy between anionic ac_2KAA(XDD) and the antibiotic. This in turn suggests that in the gas phase, as in solution, ac_2KAA(XDD) is located within the binding pocket of the antibiotic and d_6ac_2KAA(XLL) is weakly attached to the surface of the antibiotic external to the binding pocket. The gas phase structure of the binding pocket of pseudoaglycoristocetin was probed in detail by CID of dianionic mixed cluster ions with dipeptide enantiomers. With CID of $[\Psi + d_3$acAG(D) + acAG(L)–2H$]^{2-}$ and $[\Psi + d_3$acGA(D) + acGA(L)–2H$]^{2-}$, the importance of the chirality of alanine in position 1 and 2 can be assessed. In both cases a stereoselective loss of the –L-Ala ligand was observed ($I_{A+L1}/I_{A+L2} = 6.0$ and 24, respectively). This indicates that the binding pocket has retained its full

selectivity for binding to D-Ala peptides. Similar stereoselective reaction pathways were observed upon CID of mixed cluster ions comprised of Ψ and various dipeptides (see Table 3).

The positively charged mixed cluster ions were also investigated by CID. With the exception of $[\Psi + ac_2KAA(LDD) + d_6ac_2KAA(DLL) + 2H]^{2+}$, $[\Psi + ac_2KAA(DDD) + d_6ac_2KAA(LLL) + 2H]^{2+}$ none of the positively charged mixed cluster ions containing –L-Ala and –D-Ala peptide stereoisomers revealed any significant stereoselective losses upon CID. Interestingly, the stereoselective fragmentation of the two exceptions indicates that there is a preferential binding of $ac_2KAA(LXX)$ peptides to pseudoaglycoristocetin. Apparently, the stereochemical configuration of the Lysyl residue is more important than that of the C-terminal Alanyl–Alanine sequence for binding to pseudoaglycoristocetin in positive ion mode. This seems to reflect an interaction which does not involve the binding site (at least not in a manner known from solution) but nevertheless depends on the stereochemistry of the peptide ligands. The absence of any preference for binding to D-Ala peptides in positive ion mode, as observed with the vancomycin–tripeptide ions, indicates that the specific structural features that favours interaction with D-Ala peptides are lost. The most plausible explanation for this behaviour is, as with the vancomycin–tripeptide ligands, that protonation of the anionic C-terminus of the peptide ligand occurs in positive ion mode.

An interesting example of the difference in fragmentation between positively and negatively charged mixed clusters are the reaction pathways of the four ions: $[\Psi + acAA(DD) + acGA(D) \pm 2H]^{2-/2+}$ and $[\Psi + AA(DD) + GA(L) \pm 2H]^{2-/2+}$. CID of $[\Psi + acAA(DD) + acGA(D) \pm 2H]^{2-/2+}$ resulted in a preferential loss of anionic acAA(DD) in negative ion mode and protonated acAA(DD) in positive ion mode ($I_{\Psi+acGA(D)}/I_{\Psi+acAA(DD)} = 3.5$ in positive ion mode and 2.5 in negative ion mode). This indicates that acGA(D) has a higher binding affinity to Ψ in the gas phase. To test whether the preferential loss of singly charged acAA(DD) was a true stereochemical effect and not an effect caused by differences in gas phase acidity or proton affinity of the peptide ligands, CID was performed on cluster ions containing Ψ, acAA(DD) and acGA(L). The mixed cluster $[\Psi + AA(DD) + GA(L)–2H]^{2-}$ fragments by a preferential loss of anionic acGA(L) ($I_{\Psi+acAA(DD)}/I_{\Psi+acGA(L)} = 11$), showing that it is indeed the stereochemical configuration of the ligands that determines the fragmentation pathways in negative ion mode. In positive ion mode, however, the predominant loss was still protonated acAA(DD) ($I_{\Psi+acAA(DD)}/I_{\Psi+acGA(L)} = 0.3$). Consequently, dissociation of the positively charged cluster ions is not determined by the stereochemical configuration of the peptide ligands. To test whether the preferential loss of protonated acAA(DD) from $[\Psi + acAA(DD) + acGA(D) + 2H]^{2+}$ and $[\Psi + AA(DD) + GA(L) + 2H]^{2+}$ could be ascribed to acAA(DD) having a higher proton affinity than acGA(D/L), their relative proton affinities were determined by the kinetic method. The relative abundance of the product ion $[acAA(DD) + H]^+$ was higher than $[acGA(D) + H]^+$ in the CID spectrum of the protonated dimer $[acGA(D) + acAA(DD) + H]^+$. Consequently, the proton affinity of acAA(DD) is higher than that of acGA(D/L). This indicates that breakdown of $[\Psi + AA(DD) + GA(L) + 2H]^{2+}$ and $[\Psi + acAA(DD) + acGA(D) + 2H]^{2+}$ is primarily determined by the difference in proton affinity between AA(DD) and GA(D/L). Likewise, the dominating type of fragmentation pathway (i.e. loss of protonated ligand versus loss of neutral ligand) of the positively charged mixed cluster ions is in agreement with the relative order of proton affinities of the ligands. For example, ac_2KAA ligands are only lost as protonated species and they have the highest proton affinities of the peptide ligands. In contrast, the dipeptides are predominantly lost as neutral species and they have the lowest proton affinities of the peptide ligands.

The decomposition of $[\Psi + acAA(DD) + acGA(D)–2H]^{2-}$ indicates that pseudoaglycoristocetin preferentially binds acGA(D) compared to acAA(DD). In solution, ristocetin and vancomycin have higher binding affinity to acAA(DD) than to acGA(D). Whereas pseudoaglycoristocetin surprisingly have higher binding affinity to acGA(D) than to acAA(DD) [49]. It is notable that such subtle differences in solution binding affinity

are reflected by the gas phase dissociation of a pseudoaglycoristocetin mixed cluster ion.

4.3. Ristocetin mixed cluster ions

Compared to the pseudoaglycone (Ψ), there is a distinct difference in the stereoselective fragmentation of the negatively charged ristocetin mixed cluster ions, since these ions do not display the same preference for binding to –D-Ala peptides as the pseudoaglycone does. The breakdown of [R + ac_2KAA(DDD) + d_6ac_2KAA(LLL)–2H]$^{2-}$ and [R + ac_2KAA(LDD) + d_6ac_2KAA(LLL)–2H]$^{2-}$ occurs by a preferential loss of [ac_2KAA(DDD)–H]$^-$ and [ac_2KAA(LDD)–H]$^-$, respectively. The sugar residues on the ristocetin molecule are not directly involved in the interaction with the peptide ligand in solution. It appears, however, that they in some way have a very strong influence on the interaction between the antibiotic and the peptide ligands in the gas phase. Wu et al. [50] have shown that noncovalent complexes are likely to form collapsed structures in the gas phase. This means that the polar regions, which are hydrogen bonded to solvent molecules will fold up in the gas phase and form hydrogen bonds with other polar regions of the noncovalent complex. The additional saccharide units on ristocetin constitute a flexible polar moiety which may interact favorably with the peptide ligand(s) and/or with parts of the polar regions of the binding pocket and thus alter the selectivity of the binding pocket as known from solution.

The positively charged ristocetin mixed cluster ions containing ac_2KAA ligands show a similar stereoselective fragmentation as the pseudoaglycone. Apparently the presence of the additional saccharide units on ristocetin does not affect the preference for binding to ac_2KAA ligands having an L-Lysyl configuration.

4.4. Vancomycin, aglycovancomycin, and aglycovancomycinmethylester mixed cluster ions

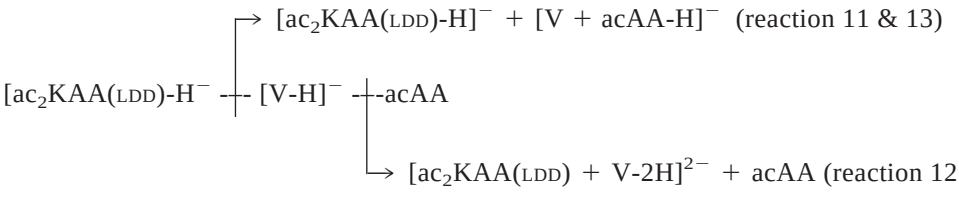
For all dianionic vancomycin and aglycovancomycin mixed clusters containing ac_2KAA(XDD) and d_6ac_2KAA(XLL) there is a pronounced preferential loss of anionic d_6ac_2KAA(XLL) (see Table 2). Such a stereoselective charge separation fragmentation is also observed for the pseudoaglycoristocetin mixed cluster ions and is in agreement with what would be expected if the ions are transferred from solution into gas phase with the ac_2KAA(XDD) ligand located within the binding site (i.e. specifically bound) and the d_6ac_2KAA(XLL) ligand weakly attached external to the binding site. The stereoselectivity of the neutral loss reactions of the clusters with the composition [d_6ac_2KAA(XLL) + V + ac_2KAA(XDD)–2H]$^{2-}$ is remarkably high since the only product ions observed are [V + ac_2KAA(XDD)–2H]$^{2-}$. Thus, the preference for binding to ac_2KAA(XDD) peptides is particularly pronounced in the neutral loss reactions. The reason for the high stereoselectivity in such neutral loss reactions is not yet clear.

So the tripeptides d_6ac_2KAA(XLL) are lost both as neutral species and as anionic species upon CID of clusters with the composition [V + ac_2KAA(XDD) + d_6ac_2KAA(XLL)–2H]$^{2-}$. This means the gas phase acidity (ΔH_{ACID}) of the tripeptides is sufficiently high to abstract a proton from the mixed cluster ions. Such a neutral loss reaction, which is observed for dianionic vancomycin and aglycovancomycin mixed cluster ions and not for the corresponding pseudoaglycoristocetin and ristocetin cluster ions, is most likely due to the presence of a mobile proton in the former ions. Assuming that the charge sites in dianionic mixed cluster ions are the C-termini, then dianionic vancomycin and aglycovancomycin mixed clusters contain three C-termini of which only two are anionic (deprotonated). The corresponding dianionic ristocetin antibiotics mixed clusters contain only two C-termini (since the C-terminal of ristocetin is methylated) which are both anionic. To further investigate the neutral loss reaction, the fragmentation of a aglycovancomycinmethylester mixed cluster ion was studied. By methylating the C-terminus on aglycovancomycin, the charge sites in dianionic mixed cluster ion must be expected to be analogous to ristocetin mixed cluster ions. Upon CID of [a$VOCH_3$ + ac_2KAA(LDD) + d_6ac_2KAA(LLL)–2H]$^{2-}$ no neutral loss of a peptide ligand was observed and a higher degree of stereoselectivity than the corresponding aglycovancomycin

mixed cluster ion was observed. This means the mobile proton plays a significant role in the dissociation of vancomycin and aglycovancomycin mixed cluster ions.

CID of $[d_6ac_2KAA(DDD) + V + ac_2KAA(LDD)–2H]^{2-}$ shows a preferential loss of $[d_6ac_2KAA(DDD)–H]^-$, which is consistent with the order of intersection energies obtained for the (1:1) complexes: $[V + ac_2KAA(LDD)–2H]^{2-}$ (E_i = 3.1 eV) and $[V + ac_2KAA(DDD)–2H]^{2-}$ (E_i = 2.6 eV) [note the only neutral loss observed is loss of $ac_2KAA(DDD)$]. The charge separation reaction of $[V + ac_2KAA(LDD) + d_6ac_2KAA(LLL)–2H]^{2-}$ occurs with a lower degree of stereoselectivity than that of $[V + ac_2KAA(DDD) + d_6ac_2KAA(LLL)–2H]^{2-}$, although the intersection energies indicate that $ac_2KAA(LDD)$ has the highest vancomycin binding affinity. The explanation might be that since $d_6ac_2KAA(LLL)$ has a higher gas phase acidity than $ac_2KAA(LDD)$, $d_6ac_2KAA(LLL)$ competes more effectively with $ac_2KAA(LDD)$ than with $ac_2KAA(DDD)$ for the proton. When the difference between the gas phase acidities ($\Delta\Delta H_{ACID}$) of two ligands increases, then it may dominate over the chiral effect. This is clearly seen in the dissociation of vancomycin mixed cluster ions containing a dipeptide and a tripeptide ligand.

The charge separation reactions occur without any stereoselectivity, whereas the neutral loss reactions are clearly dependent upon the chirality of the ligands. The "mobile" proton in such clusters is fixed to the C-terminus of the dipeptide, since this ligand has the highest gas phase acidity. Consequently, the dipeptide carrying a neutral C-terminus cannot interact specifically with vancomycin, which explains the lack of stereoselectivity in the charge separation reactions. Since the dipeptide has sequestered the mobile proton, vancomycin $[V–H]^-$ can interact strongly and stereospecifically with $[ac_2KAA(LDD)–H]^-$, but not with $[ac_2KAA(LLL)–H]^-$. The much lower gas phase vancomycin binding affinity to $ac_2KAA(LLL)$ shuts off the neutral loss reaction for $[acAA(DD) + V + ac_2KAA(LLL)–2H]^{2-}$ [reaction (16)]. The charge separation reaction and the neutral loss reaction observed for $[acAA(DD) + V + ac_2KAA(LDD)–2H]^{2-}$ [reactions (11) and (12)] and $[acAA(LL) + V + ac_2KAA(LDD)–2H]^{2-}$ [reactions (13) and (14)] is then rationalized by cleavage at the hypothetical hydrogen bonds (dotted lines) shown in the following:



5. Conclusion

From the present study it can be concluded that in some cases the known solution behaviour of noncovalent complexes formed between vancomycin antibiotics and peptide ligands is directly reflected in the stereoselective fragmentation of dianionic mixed cluster ions comprised of an antibiotic and two stereoisomeric peptide ligands, one isotopically labeled. For dianionic vancomycin–tripeptide complexes the order of relative gas phase stabilities was found to be identical to the order of solution association constants, which were determined from relative ion abundances obtained from competition experiments. The results presented in this study strongly suggest that the structural features of the antibiotics, which are responsible for the stereoselective binding of –D-Ala peptides, are preserved in the gas phase for anionic complexes. In contrast, it appears that the positively charged complexes have lost their structural specific interaction when they are transferred from solution to gas phase. For instance, no difference in the reactivity of positively charged complexes between vancomycin and tripeptide stereoisomers could be observed. Likewise, no significant stereoselective fragmentation in the decomposition of positive-charged mixed cluster

ions was observed. This indicates that the C-terminus of the peptide ligand is protonated (neutral) in positively charged complexes which destroys the specific interaction between antibiotic and peptide ligand. Unexpectedly did the saccharide substituents on ristocetin modify the stereoselectivity for binding to –D-Ala peptides in the gas phase. Thus, the decomposition of anionic ristocetin mixed cluster ions occurred with a predominant loss of –D-Ala peptides. This seems to reflect an interaction between antibiotic and peptide ligand which does not involve the solution binding site, but nevertheless depends on the stereochemical configuration of the peptide ligand.

The stereoselective decomposition of anionic pseudoaglycoristocetin mixed cluster ions revealed that subtle differences in solution binding affinities were reflected in the gas phase. It is encouraging that such receptor–ligand binding phenomena can be observed in the gas phase, since the solvent free environment in the vacuum of a mass spectrometer offers unique possibilities for studying the role of the solvent in the interaction and the naked noncovalent complex ion provides means for studying the intrinsic nature of the interaction, which is fundamental for understanding the mechanisms of molecular recognition.

Acknowledgements

Vancomycin and ristocetin were kindly donated by Dumex (Copenhagen, Denmark) and Alpharma (Oslo, Norway), respectively. The Danish Biotechnology Program and the Danish National Research Foundation are acknowledged for financial support.

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