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Tandem mass spectrometry of specific vs. nonspecific noncovalent complexes of vancomycin antibiotics and peptide ligands

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This paper is dedicated to Yannik Hoppilliard on the occasion of her 60th birthday.

Abstract

The dianionic noncovalent complex between vancomycin and the bacterial cell-wall precursor peptide Ac_2-L-Lys-D-Ala-D-Ala (DD) has previously been shown to retain its structural specific interactions from solution into the gas phase [Int. J. Mass Spectrom. Ion Processes 188 (1999) 63]. We have now determined the destruction cross sections for this specific complex and for the nonspecific complex between vancomycin and Ac_2-D-Lys-L-Ala-L-Ala (LL). Although the gaseous nonspecific complex is only very loosely bound compared to the specific complex, their destruction cross sections are identical. This indicates that there is no difference in the compactness of their gas phase structures. Upon high-energy collisional activation ($E_{LAB} = 100$ keV) of the specific dianionic complex, electron detachment occurs and covalent bond cleavage with retainment of noncovalent bonds is observed; these reactions were not observed at low collision energy ($E_{LAB} = 300$ eV). Furthermore, we now report how small changes in the peptide binding pocket of vancomycin affect gas phase stability in a manner which parallels known solution binding affinity. The results show that Ac_2-L-Lys-D-Ala-D-Ala cannot form as strong electrostatic interactions with CDP-1 as with vancomycin (or dechlorovancomycin) in the gas phase. (Int J Mass Spectrom 219 (2002) 659–670)

Keywords: Electrospray ionization; Noncovalent complexes; Vancomycin; High-energy collision-induced dissociation; Destruction cross sections

1. Introduction

Vancomycin is a naturally occurring glycopeptide antibiotic which is active against Gram-positive bacteria. The antimicrobial effect occurs through reversible binding to bacterial cell-wall precursors with the C-terminal sequence: -L-amino acid-D-Ala-D-Ala [2]. The action of specific bacterial enzymes that would otherwise use these termini to form new cross links in the nascent bacterial cell wall is, thereby, inhibited and eventually this leads to cell death [3]. The molecular basis for antibacterial activity has been investigated through numerous studies of complexes formed between the vancomycin group antibiotics and bacterial cell-wall mimicking model peptides (for a review, see [4]). These studies have shown that the binding of a -D-Ala-D-Ala peptide ligand to vancomycin involves several intermolecular hydrogen bonds, which are formed between on the one side the carboxylate anion and amides of the peptide ligand and on the other side the backbone amides of the antibiotic (see Fig. 1(a)).

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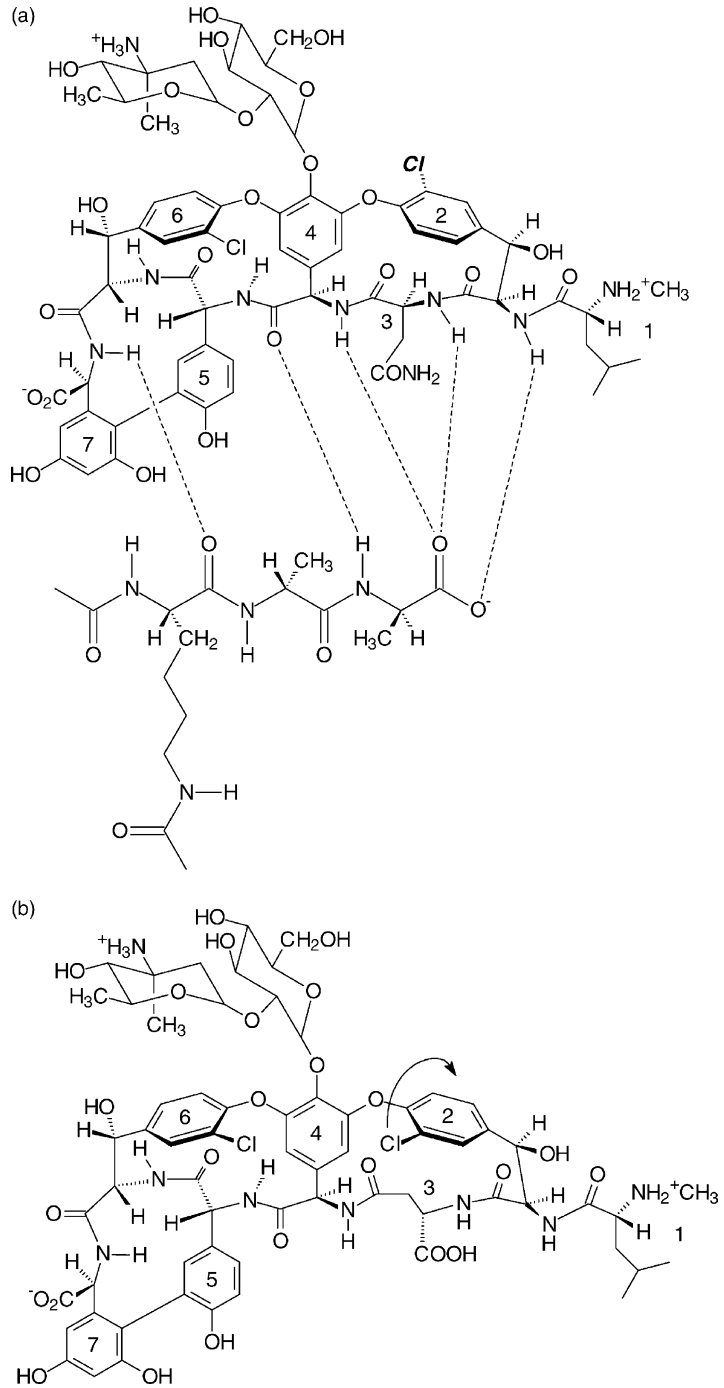

Fig. 1. Noncovalent complex formed between vancomcyin and the tripeptide cell-wall precursor analogue Ac_2-L -Lys-D -Ala-D -Ala. Dashed lines indicate hydrogen bonds. In dechlorovancomycin, a single chlorine atom (given in bold italics) has been replaced by a hydrogen atom (a); CDP-1 (crystalline degradation product 1 of vancomycin). The arrow indicates that CDP-1 exists as atropisomers which differ in the orientation of the chlorine on ring 2, it is the major isomer which is depicted here (b).

The structural features of the peptide ligand which are most critical for the binding are the two C-terminal D -Ala residues [4,5]. 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.

Electrospray ionization mass spectrometry (ESI-MS) has proved to be a useful technique for obtaining accurate solution association constants for the complexes formed between vancomycin group antibiotics and bacterial cell-wall mimicking peptide ligands [6–8]. We have previously shown that when such complexes are analyzed in negative ion mode, they retain their structural specific interactions from solution into the gas phase [1]. This finding was based upon the influence of the stereochemistry of the peptide ligand on the gas phase stability of several noncovalent complexes. Thus, in the gas phase, a high stereoselective preference for binding to -D -Ala peptides and not to -L -Ala peptides were observed. For example, the dianionic noncovalent complex between vancomycin and the nonspecific tripeptide Ac_2-D -Lys-L -Ala-L -Ala (LL) is more readily dissociated upon collisional activation than the complex where vancomycin is bound to the specific ligand Ac_2-L -Lys-D -Ala-D -Ala (DD). The difference in the ease of dissociation reflects that the nonspecific peptide has a stereochemical configuration which is incompatible with the geometry of the binding pocket of vancomycin. It may then be suggested that the complex with Ac_2-L -Lys-D -Ala-D -Ala has a more compact structure than the complex with the nonspecific tripeptide, since Ac_2-L -Lys-D -Ala-D -Ala fits the binding pocket of vancomycin perfectly; whereas the nonspecific peptide is only loosely attached to the antibiotic. The question we want to address is: “Is the gas phase structure of the specific complex more compact than the nonspecific complex?”

We have probed the compactness of the gas phase structures of the aforementioned noncovalent complexes by measuring their destruction cross sections. The destruction cross section provides direct information about the geometry of the biomolecular ion [9]. We have previously used this method to study the gas phase conformations of multiply charged lysozyme ions [9].

Furthermore, we report how small changes in the peptide binding pocket of vancomycin affects gas phase stability of noncovalent complexes in a manner which parallels known solution binding affinity.

2. Experimental

2.1. Low-energy collision experiments

Experiments were performed on a Finnigan TSQ 700 triple quadrupole mass spectrometer equipped with a nanoelectrospray ion source (MDS 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. An octapole guides the ions from the skimmer region to the first quadrupole. The temperature and voltage differences in the ESI-interface were carefully adjusted in order to provide just sufficient energy to completely desolvate the dianionic noncovalent complexes without inducing dissociation of the complexes. The conditions were: heated capillary offset +15 V, heated capillary temperature 180 °C, tube lens voltage −60 V, octapole offset +1.5 V, the potentials were maintained at constant settings throughout the entire m/z range. The noncovalent complexes were mass selected in the first quadrupole ($Q1$) and then accelerated into the collision cell ($q2$). Xenon was used as collision gas instead of argon. Only with xenon could a sufficiently high center-of-mass collision energy (E_{CM}) be obtained, i.e., a collision energy large enough to ensure that each collision leads to fragmentation. The maximum collision cell ($q2$) offset is 195 V on the Finnigan TSQ700; therefore, the highest available E_{CM} for the noncovalent complex formed between vancomycin (V) and Ac_2-L -Lys-D -Ala-D -Ala, $[V+DD–2H]^{2-}$, is 8.4 eV when argon is used as collision gas, whereas xenon gives a maximum E_{CM} of 26.2 eV. From the dissociation efficiency curve generated for $[V+DD–2H]^{2-}$ (Fig. 4), it can be seen that the product ion yield begins to saturate around 14 eV.

Thus, 14 eV is the minimum E_{CM} which is sufficiently high to ensure that each collision leads to fragmentation of the precursor ion. For the destruction cross section measurements, we used $E_{CM} = 20$ eV (i.e., $q2$ offset was +150 V) which is well above the minimum energy limit for both $[V + DD–2H]^{2-}$ and $[V + LL–2H]^{2-}$. The pressure in the collision octapole was monitored by a capacitance pressure gauge (MKS Baratron 627A). The product ions were mass analyzed in the third quadrupole ($Q3$). For the dissociation efficiency curves, the kinetic energy of the precursor ion (E_{LAB}) was varied from 0 to 235 eV corresponding to 0 to 16 eV E_{CM}. Single collision conditions were estimated by the method of Dawson [10]. Note that it is not feasible to obtain accurate dissociation threshold energies from the dissociation efficiency curves since we have not made any corrections for the kinetic shift and the ions generated in the electrospray ionization interface have an unknown internal energy distribution. Although true dissociation threshold energies cannot be obtained for the noncovalent antibiotic–peptide complexes, their dissociation efficiency curves still provide valuable information such as the order of threshold energies for the complexes. This order is determined from the relative positions of the dissociation curves. The solutions for ESI-MS analysis were prepared by diluting aliquots of 2.0 mM stock solutions of vancomycin and tripeptide ligands with 5.0 mM ammonium acetate buffer (pH 5.1) to yield a concentration of 50 μM. Vancomycin hydrochloride and its derivatives were kindly donated by Torben Koch from Dumex (Copenhagen, Denmark). The cell-wall precursor analog Ac_2-L -Lys-D -Ala-D -Ala was purchased from Sigma and used without further purification. The enantiomer of the cell-wall precursor analog Ac_2-D -Lys-L -Ala-L -Ala was synthesized by solid phase peptide synthesis using a standard Fmoc protocol [1].

2.2. Destruction cross sections

The total destruction cross sections for the ions were derived from the exponential dependence of the precursor ion beam intensity I on the thickness (μ) of the target gas, $I(\mu) = I(0)e^{-\sigma\mu}$. The destruction cross section σ is the sum of all loss processes for the precursor ion including fragmentation and scattering. $I(0)$ is the initial intensity of the precursor ion signal (i.e., without collision gas in the collision cell), $I(\mu)$ is the intensity of the precursor ion signal after addition of the collision gas, at target thickness μ. The target thickness was calculated assuming a uniform collision gas density in the collision cell with the following parameters for low-energy collision experiments: gas temperature 70 °C (i.e., manifold temperature), length of collision cell 18.51 cm. The path length of ions in the octapole is somewhat longer than 18.51 cm due to the radial motion. However, generally, the correction for this is only a few percent [11]. In the high-energy collision experiments, the length of the collision cell was 3.0 cm, collision gas temperature was 20 °C.

2.3. High-energy collision experiments

A detailed description of our instrumental set-up was given previously [9]. Briefly, a home–made electrospray ion source has been mounted on an existing electrostatic accelerator of the isotope separator type. The electrospray ion source (including rotary pumps and turbo molecular pumps) is mounted on the high-voltage platform of the accelerator. The first stages of the ESI-interface are essentially identical to those of the Finnigan TSQ700, and hence similar ion source conditions were used to observe the noncovalent complexes. The instrument operates at an acceleration potential of 50 kV (the kinetic energy of $[V + DD–2H]^{2-}$ is then 100 keV and $E_{CM} = 110$ eV, when H_2 is the target gas). The precursor ions are mass-selected with a magnet then collisionally activated in a 3.0 cm collision cell. A capacitance pressure gauge measured the collision gas pressure. The ions exiting the cell are separated according to their energy-to-charge ratio (E/z) by a 180° hemispherical electrostatic analyzer. The transmitted ions are detected by a channeltron operating in the particle counting mode. In this way, mass-analyzed ion kinetic energy (MIKE) spectra could be obtained.

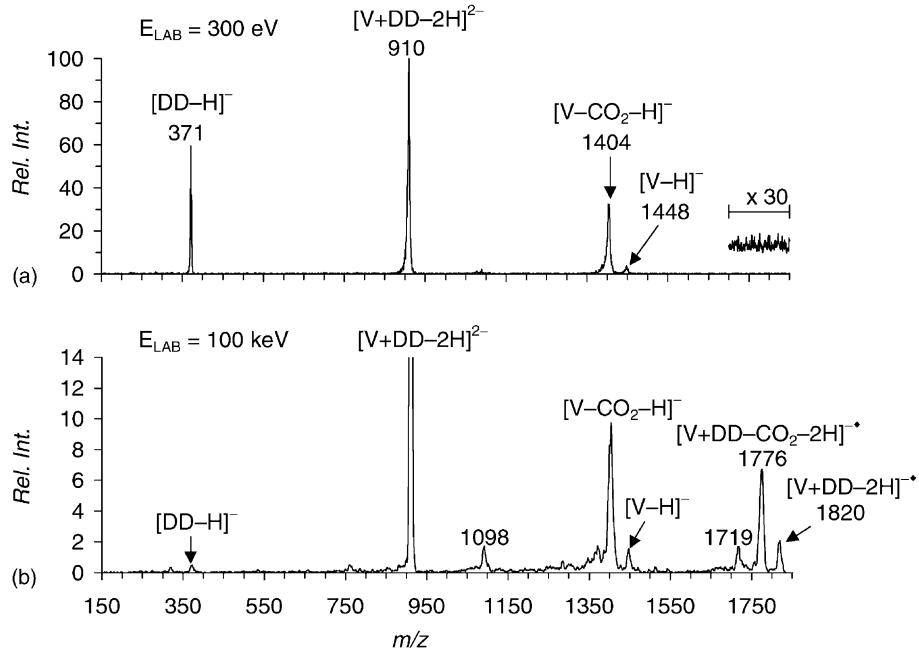

Fig. 2. CID spectra of the dianionic complex formed between vancomycin (V) and Ac_2-L -Lys-D -Ala-D -Ala (DD) obtained at (a) low-energy, i.e., $E_{LAB} = 300$ eV, collision gas Xe, pressure 0.12 mTorr and (b) high-energy, i.e., $E_{LAB} = 100$ keV, collision gas H_2, pressure 0.40 mTorr.

3. Results and discussion

3.1. Product ion (MS/MS) spectra

Fig. 2a and b shows the collision-induced dissociation (CID) spectra of the dianionic noncovalent complex $[V + DD–2H]^{2-}$ obtained at collision energies (E_{LAB}) of 300 eV and 100 keV. At low collision energy (Fig. 2(a)), the complex dissociates by a charge separation process into a singly-charged tripeptide $[DD–H]^-$ (*m/z* 371) and singly-charged vancomycin $[V–H]^-$ (*m/z* 1448) (reaction 1). The vancomycin product ion undergoes further fragmentation and CO_2 is lost to give the decarboxylated product ion $[V–H–CO_2]^-$ at *m/z* 1404 (reaction 2).

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

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

At high collision energy Fig. 2(b)) reactions (1) and (2) are also observed. In addition, the high collision energy CID spectrum contains three distinct peaks at *m/z* 1719, 1776, and 1820 which are not present in the low collision energy CID spectrum. The peak at *m/z* 1820 corresponds to the anionic radical $[V + DD–2H]^{\bullet -}$ which is formed by electron detachment from the precursor ion (reaction 3). Loss of CO_2 from $[V + DD–2H]^{\bullet -}$ occurs readily and this process yields the decarboxylated anionic noncovalent radical at *m/z* 1776 (reaction 4).[1]

$$[V + DD–2H]^{2-} \rightarrow [V + DD–2H]^{\bullet -} + e^- \quad (3)$$

$$[V + DD–2H]^{\bullet -} \rightarrow [V + DD–2H–CO_2]^{\bullet -} + CO_2 \quad (4)$$

The peak at *m/z* 1719 can be explained by loss of a tert-butyl radical ($C_4H_9^{\bullet}$) from the sidechain of

[1] The decarboxylated anionic radical $[V + DD–2H–CO_2]^{\bullet -}$ at *m/z* 1776 may also be generated by loss of $CO_2^{\bullet -}$ from $[V + DD–2H]^{2-}$. Note that although $CO_2^{\bullet -}$ is metastable with respect to electron detachment, it has been observed in high-energy collisions of carboxylate anions [12]. However, we did not observe $CO_2^{\bullet -}$ in our experiments.

the N-terminal *N*-methyl-D -leucin in vancomycin. This reaction is governed by the radical character of $[V + DD–2H–CO_2]^{\bullet -}$. The peak at *m*/*z* 1098 corresponds to decarboxylated aglycovancomycin (i.e., vancomycin without the disaccharide). This reaction is also observed at low collision energy CID albeit at lower abundance. Note that the high-energy CID spectrum underrepresent the abundances of low mass ions relative to those of the high mass ions. In order to compare relative ion abundances, the peak areas of interest must be divided by the electrostatic analyzer voltage at which the ions were transmitted. This partly explains why the peak area of $[DD–H]^-$ in Fig. 2(b) is considerably smaller than the sum of the peak areas of $[V–H–CO_2]^-$ and $[V–H]^-$. However, the corrected ion abundance ratio $[DD–H]^-/([V–H–CO_2]^- + [V–H]^-)$ is only 15%, suggesting that a significant part of $[V + DD–2H–CO_2]^{\bullet -}$ dissociates into $[V–H–CO_2]^-$ and $DD^{\bullet}$.

Decarboxylation of the anionic noncovalent complex (i.e., reaction 4) and the putative loss of a tert-butyl radical ($C_4H_9^{\bullet}$) are both reactions in which covalent bond cleavage occurs without dissociation of the noncovalent bonds in the complex. Breakage of covalent bonds with retainment of the noncovalent complex has been observed in a number of cases [13–19]. The competition between noncovalent and covalent dissociation was investigated in detail for cyclodextrin–peptide complexes [18] and DNA duplexes [17]. Both studies demonstrated that slow heating favors covalent breakage. However, in our case a different situation prevails, since covalent bond cleavage occurs exclusively in the anionic radical, which is only formed under high-energy collisional activation (i.e., fast heating). Collisional electron detachment (reaction 3) occurs on an extremely short time scale. The dianionic noncovalent complex with a mass of 1820 Da and a kinetic energy of 100 keV moves with a speed of 1.0×10^5 m/s, which gives a collision interaction time of ∼10 fs, assuming an interaction length of 10 Å [20]. Since the vibrational periods of C–C, C–O, and C–N bonds ($\sim 3 \times 10^{-14}$ s) and of C–H, O–H, and N–H bonds ($\sim 1 \times 10^{-14}$ s) are commensurable with the interaction time, the electron detachment process is nearly vertical. Hence, the anionic radical is most likely formed with several quanta of vibrational excitation determined by the Franck–Condon factors, which is then sufficient to cause the losses of carbon dioxide and the tert-butyl radical. But the internal energy is still smaller than that required to dissociate the noncovalent bonds [21].[2] Note that the radical nature of $[V + DD–2H]^{\bullet -}$, most likely, causes the activation energies for the aforementioned covalent bond cleavages to be lower than those of the corresponding cleavages in the even electron counterpart.

It is noteworthy that only a few types of fragment ions are observed at both high and low collision energy. In particular, no ions corresponding to cleavage of any of the peptide bonds are discernible. This somewhat unusual lack of reactivity for a peptide was also observed in positive ion mode [22].[3] The resistance of vancomycin towards fragmentation is undoubtedly due to the extensive cross-linking of the side chains of the amino acid residues. The cross-linking gives vancomycin a rigid tricyclic structure and, within this structure, at least two covalent bond cleavages are required in order to produce two separate fragment ions.

3.2. Destruction cross sections

Fig. 3 shows the attenuation of the ion beam intensity as a function of the collision gas thickness. From the slopes of the fitted lines, the destruction cross sections of the noncovalent complexes are directly obtainable. At low collision energy ($E_{LAB} = 300$ eV), the destruction cross section for the dianionic noncovalent complex formed between Ac_2-L -Lys-D -Ala-D -Ala and vancomycin is identical (within experimental

[2] A similar observation was made for gaseous monoprotonated hydrated amino acids. In this case, collisional electron transfer to O_2 was observed without dissociation of the radical dicationic water–amino acid cluster [21].

[3] High and low energy CID of diprotonated vancomycin caused predominantly cleavage within the sugar moiety (Jørgensen, Hvelplund, and Roepstorff, unpublished results). Similar observations were made upon collisional activation of monoprotonated vancomycin [22].

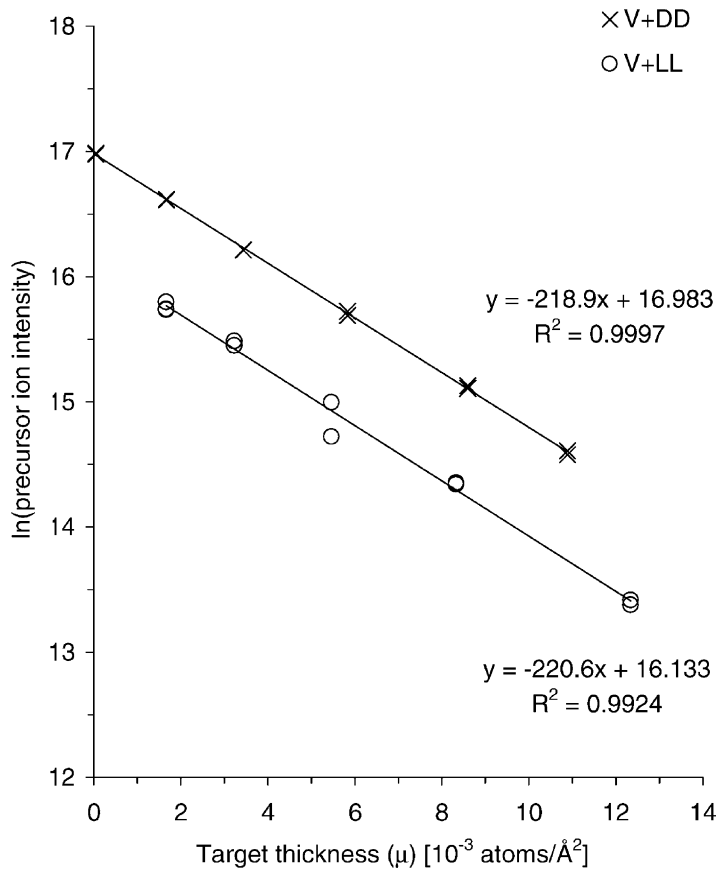

Fig. 3. Exponential fit to the measured beam intensity vs. target thickness for $[V + DD–2H]^{2-}$ (cross) and $[V + LL–2H]^{2-}$ (open circle). The collision gas was Xe and the collision energy (E_{LAB}) was 300 eV. The total destruction cross section is obtained from the slope of the fitted line.

error) to that of the complex between vancomycin and Ac_2-D -Lys-L -Ala-L -Ala (Table 1). This indicates that there is no difference in the compactness of their gas phase structures. Our previous investigations have shown that the gaseous complex between vancomycin and Ac_2-L -Lys-D -Ala-D -Ala (i.e., $[V + DD–2H]^{2-}$) has retained its structural specific interactions from solution [1]. Such a gas phase structure would be expected to be rather compact due to the high degree of complementarity between the Vander Waals surfaces in the binding interface and the favorable geometry of the intermolecular hydrogen bonds. In contrast, the peptide binding pocket of vancomycin have completely the wrong shape for a favorable

Table 1
Destruction cross sections (σ) for noncovalent complexes between vancomycin (V) and tripeptide ligands Ac_2-L -Lys-D -Ala-D -Ala (DD) and Ac_2-D- Lys-L- Ala-L -Ala (LL) determined at low[a] and high[b] collision energy

Ions	σ [Å^2]
$[V + DD–2H]^{2-}$	220.6 ± 1.5, **210.7 ± 1.3**
$[V + LL–2H]^{2-}$	221.0 ± 1.6
$[V + DD + 2H]^{2+}$	**200.7 ± 2.8**
$[V + 2H]^{2+}$	**152.4 ± 1.0**

Numbers in bold were obtained at high collision energy. The uncertainty is ± standard deviation of six measurements.
[a] E_{LAB} = 300 eV, target = Xe, E_{CM} = 20 eV.
[b] E_{LAB} = 100 keV, target = H_2, E_{CM} = 110 eV.

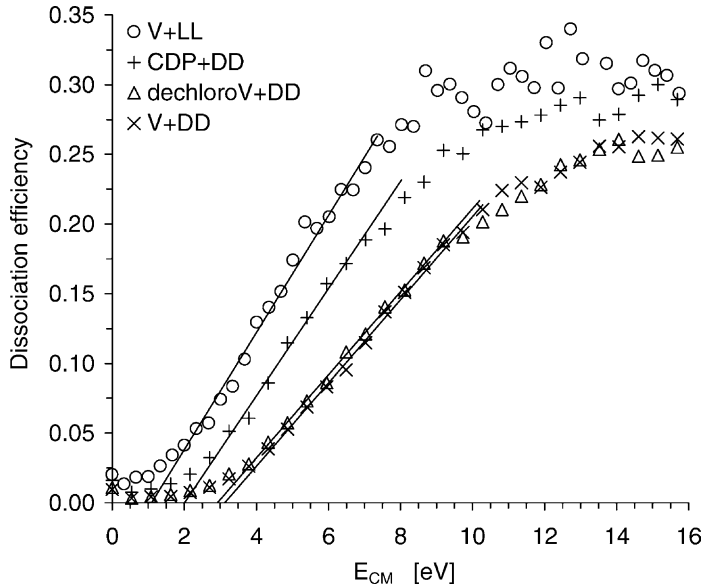

Fig. 4. Dissociation efficiency curves of four dianionic noncovalent complexes. The complexes are: $[V + LL–2H]^{2-}$ (open circle), $[CDP + DD–2H]^{2-}$ (plus), $[dechloroV + DD–2H]^{2-}$ (open triangle), and $[V + DD–2H]^{2-}$ (cross). The ions were collided with Xe at single collision conditions (pressure 0.080 mTorr) at various collision energies. The *y*-axis represents the dissociation efficiency, which is the abundance of the tripeptide product ion in proportion to the sum of the abundances of the precursor ion and tripeptide production.

interaction with Ac_2-D -Lys-L -Ala-L -Ala as evidenced by the significant lower dissociation threshold energy of $[V + LL–2H]^{2-}$ relative to that of $[V + DD–2H]^{2-}$ (Fig. 4). The finding that the two complexes have identical destruction cross sections was thus somewhat surprising, since the different dissociation thresholds reflect that the noncovalent bonding interactions in the two complexes certainly are dissimilar. An explanation for this behavior may be that although the binding pocket of vancomycin does not support optimal binding to Ac_2-D -Lys-L -Ala-L -Ala, the flexibility of this tripeptide allows it to adopt a conformation which still generates a rather compact noncovalent complex. It is, thus, likely that the same number of hydrogen bonds may exist in both complexes and this leads to compact gas phase structures in both cases. But the dissociation thresholds will still be different, since the energy of a hydrogen bond is critically dependent upon its exact geometry [23]. Such a picture is in accordance with the recent knowledge on the gas phase structures of peptides, where both experiments and calculations have demonstrated that it is energetically favorable to adopt fairly compact gas phase structures with extensive hydrogen bonding [24,25]. Furthermore, Wu et al. [26] have shown that the noncovalent complexes between a protein and various peptide ligands are likely to form collapsed structures in the gas phase. This means that the polar regions, which are hydrogen bonded to solvent molecules in solution, will fold up in the gas phase and form hydrogen bonds with other polar regions within the noncovalent complex. A similar observation was made for the noncovalent complex between cytochrome *c* and cytochrome b_5. For a given charge state, the collision cross section of this complex was found to be only slightly greater than that of its monomers, also suggesting a collapsed and compact gas phase structure [27].

The destruction cross sections obtained at high collision energy ($E_{LAB} = 100$ keV) are listed in Table 1. There is fairly good agreement between the destruction

cross sections for $[V+DD–2H]^{2-}$ obtained at low and high collision energy.[4] Unfortunately, the abundance of the nonspecific complex (i.e., $[V+LL–2H]^{2-}$) was too low to measure its destruction cross section using the accelerator mass spectrometer. We, then, determined the destruction cross section for the doubly protonated complex, which is also known to be nonspecific in the gas phase [1]. Unexpectedly, the destruction cross section for this complex was found to be somewhat lower (∼10 Å^2) than that of the dianionic complex. We have previously shown that the structural specific interactions responsible for the stereoselective binding of D -Ala-D -Ala peptides are not retained in the gaseous doubly protonated complex [1]. This behavior was explained by a protonation of the anionic C-terminus of the peptide ligand which leads to a weakening of the hydrogen bonds which are otherwise formed between the anionic C-terminus and the amide hydrogens in vancomycin. In this way, the network of hydrogen bonds which is responsible for stereoselective binding of D -Ala-D -Ala is destroyed in the doubly protonated complex. Thus, the carboxylic groups are most likely neutralized in the cationic complex and the two ionizing protons are then located at the two amino groups in vancomycin which are the most basic sites in the complex. This charge configuration has possibility for a relative large intercharge distance to relieve Coulombic repulsion, since the charged disaccharide moiety is quite flexible. In the dianionic complex, the charge sites are most likely the carboxylate anions. Also this configuration gives a relative large intercharge distance (∼12 Å in the solution structure [28]). Hence, the localization of charges is quite different in the cationic and anionic noncovalent complexes and this leads to the observed differences in their destruction cross sections. A similar observation was reported in an ion mobility study of protonated vs. deprotonated cytochrome *c* [25]. In this case, the collision cross section of the −4 charge state was slightly higher than that of the +4 charge state. At intermediate charge states ($4 < n < 10$), the cross sections of the anions became less than those of the cations.

Ion mobility measurements have demonstrated that for a given charge state of a gaseous protein, there may exist several conformers having different collision cross sections [25]. With our method, the presence of two or more conformers with different destruction cross sections will be revealed by a multiterm exponential dependence of the beam intensity on the target thickness. However, in all our experiments, the ion beam intensity was found to undergo a simple attenuation giving a good fit to a single-term exponential decline with increasing collision gas thickness. Thus, our results show that if a population of conformers exists for a given noncovalent complex then their destruction cross sections are quite similar.

3.3. Dissociation thresholds

We have previously determined the influence of stereochemistry of the peptide ligand on the gas phase stability of noncovalent complexes between peptide stereoisomers and vancomycin antibiotics. Now we extend our studies to investigate the effect of changes in the binding pocket of vancomycin on the gas phase stability of its noncovalent complexes. Two derivatives of vancomycin called crystalline degradation product 1 (CDP-1) and dechlorovancomycin were selected for the present study. In CDP-1, the conformation of the binding pocket has changed so that binding to Ac_2-L -Lys-D -Ala-D -Ala in solution is dramatically decreased (the solution association constant K_A is reduced by more than a factor of 1000) [29]. In dechlorovancomycin, the binding affinity is also reduced but only with a factor of ∼2 relative to that of vancomycin [30]. Note that we have previously shown that when solutions containing vancomycin, dechlorovancomycin, and Ac_2-L -Lys-D -Ala-D -Ala are analyzed with ESI-MS then the relative ion abundances of the free antibiotics and their noncovalent

[4] The good correlation between the destruction cross sections obtained at low and high energy may be explained in a model, where energy is transferred to individual projectile atoms in semielastic collisions with the target atom. When the interaction between the atoms is described by screened Coulomb potentials, it is found that the energy required for bond breaking (∼5 eV) is transferred at similar distances for high-energy collisions with H_2 and low-energy collisions with Xe.

complexes accurately reflect their known solution binding affinities [7]. However, their relative gas phase stabilities have not been addressed before.

CDP-1 is formed by hydrolysis of vancomycin under weakly acidic conditions. An intramolecular rearrangement and hydrolysis of the primary amide converts the asparagine residue in vancomycin into an iso-aspartate, and in the course of the rearrangement a chlorinated aromatic ring flips over by 180° (see Fig. 1(b)). In solution, CDP-1 exists as atropisomers (i.e., rotational isomers) which differ in the orientation of the chlorine on ring 2. Both isomers exhibit very low affinity towards peptide ligands ($K_A \ll 1000\,M^{-1}$) and they have no antimicrobial activity. The decreased binding affinity to D -Ala-D -Ala peptides is thought to originate from an extra methylene group in the peptide backbone, slightly elongating the carboxylate binding pocket [29]. The difference, this makes to the shape of the binding site, is small, and it is possible to fit the peptide into the binding site; yet the structure does not preserve the right distances for optimal hydrogen-bonding network. Fig. 4 shows that the dianionic complex between CDP-1 and Ac_2-L -Lys-D -Ala-D -Ala dissociates more readily than the complex between vancomycin and Ac_2-L -Lys-D -Ala-D -Ala. As stated earlier, the geometry of the binding pocket of vancomycin is designed for an optimal hydrogen-bonding network with peptides having the C-terminal sequence -D -Ala-D -Ala. Such intermolecular hydrogen bonds are highly distance dependent and directional, with preference for the hydrogen atom lying in the plane of carbonyl bond and along the direction of oxygen's lone pairs [23]. In CDP-1, the conformation of the binding pocket is changed to a less optimal configuration for the formation of strong intermolecular hydrogen bonds. Thus, Ac_2-L -Lys-D -Ala-D -Ala cannot form as strong electrostatic interactions with CDP-1 as with vancomycin and this leads to the observed differences.

In dechlorovancomycin, a chlorine on the aromatic ring of residue 2 has been replaced by a hydrogen atom (see Fig. 1(a)). This chlorine is located on the backside of the binding pocket and it is not directly involved in the interaction with the peptide ligand. It was, therefore, somewhat surprising that dechlorovancomycin showed decreased binding in solution [30]. It was speculated that the reduced binding affinity was due to an increased mobility of the ring which in turn weakened the intermolecular hydrogen bonding to the peptide. Our gas phase measurements support this idea, since removal of the chlorine slightly destabilizes the noncovalent interactions (Fig. 4). However, other factors than weaker intermolecular hydrogen bonds may also contribute to the decreased affinity observed in solution and these factors will not be present, when the complex is examined in the gas phase. NMR spectroscopy studies of free vancomycin have shown that the peptide bond between residues 2 and 3 rotates, so that the amide proton of residue 3 spends some of its time on the back face of vancomycin (note, in this conformation the carboxylate anion binding pocket is *not* present) [31]. During the rotation, the amide proton passes under the aromatic ring of residue 2 and it has been suggested that this motion may play a role in desolvating the binding site prior to ligand binding [32]. It is likely that removal of the chlorine affects the barrier to the rotation of the peptide bond and perhaps also the conformational equilibria. If the stability of the nonbinding conformer is slightly increased, when chlorine is removed, then the overall binding affinity in solution will be reduced but such an effect cannot be revealed from gas phase stability measurements.

In solution, the interplay between solvent–solvent, solvent–solute, and solute–solute interactions are responsible for the magnitude of the association constant for complex formation between two solutes. Hydrophobic interactions are the result of solvent–solvent interactions being far more favorable than solvent–solute interactions. It is this effect that causes hydrocarbon molecules to spontaneously cluster, when they are placed in water, so as to minimize the hydrocarbon surface that is exposed to the water. It is well known that hydrophobic interaction are important for the stabilization of many biomolecular complexes [33,34]. Since hydrophobic interactions cannot a priori contribute to stabilization of a solvent-free noncovalent complex then one should, in general, not expect any correlation between gas phase

stability and solution binding affinity. This has also been observed in several cases [26,35–37]. However, the dianionic noncovalent complexes in our study exhibit a good correlation between their gas phase stability and known solution binding affinity. The ease of dissociation of such a gaseous complex depends solely on the intermolecular electrostatic binding energy. In solution, the ionic and neutral C=O···H–N intermolecular hydrogen bonds play a decisive role in the stabilization of the vancomycin–tripeptide association. Since the hydrogen bond is an electrostatic interaction which is also favorable in the gas phase, this may be the main reason for the good correlation between gas phase stability and solution binding affinity. However, such a correlation presupposes that the structural specific intermolecular hydrogen bonds are retained from solution into the gas phase. An additional requirement is that polar regions of the complex, which are hydrogen bonded to water molecules in solution should not contribute significantly to the intermolecular electrostatic interaction energy in the gas phase (i.e., nonspecific intermolecular interactions must be weak). We believe that the structural ridigity of vancomycin is very important for fulfilling these requirements. First, it preserves the geometry of the binding pocket of vancomycin, so that an optimal hydrogen-bonding network with peptides having the C-terminal sequence -D -Ala-D -Ala also exists in the gas phase. Second, the structural rigidity of vancomycin prohibits the formation of energetically favorable nonspecific intermolecular interactions. There are several other studies which indicate that when electrostatic interactions play a major role for complex formation in solution then these interactions may be retained in the gas. When such intrinsic features of the interaction may be probed by CID, as demonstrated for DNA duplexes [15,19,38,39], the heme binding to variant forms of proteins [40] and in other complexes [41–43].

If the interaction partners in a gaseous noncovalent complex each carry one or more charges then the intermolecular binding energy will be reduced by the electrostatic repulsion and the overall dissociation process may become exothermic [44]. The repulsion also imposes a reverse activation barrier (i.e., an activation energy for the reverse reaction) for dissociation by charge separation. The height of the reverse activation barrier is to a good approximation equal to the Coulomb repulsion energy, ϵ_r, which can be calculated by: $\epsilon_r = 14.4\,\text{eV}\,\text{Å}/r$, where r is the intercharge distance in Å. In this simple model the charged groups are treated as point charges and there is no shielding provided by the peptide (i.e., the dielectric constant, ε, is 1.0) and no intramolecular hydrogen bonds are formed in the monomers concurrently as the intermolecular bonds are ruptured. Note that in water the coulombic repulsion will be reduced by the dielectric constant of water ($\varepsilon \sim 80$). The intercharge distance in the gaseous dianionic complex between vancomycin (and dechlorovancomyin) and Ac_2-L -Lys-D -Ala-D -Ala is assumed to be close to 12 Å. The intercharge distance in the nonspecific complexes is not known. The reverse activation barrier for the dissociation of the specific gaseous complexes is then estimated to ∼1.2 eV. Measurement of the kinetic energy release for dissociation of specific vs. nonspecific complexes would be of interest in order to elucidate the structural differences between these complexes.

4. Conclusion

We have determined the destruction cross section for the dianionic complex between vancomycin and the bacterial cell-wall precursor peptide Ac_2-L -Lys-D -Ala-D -Ala and that of the complex between vancomycin and Ac_2-D -Lys-L -Ala-L -Ala. In the former complex, the structural specific interactions responsible for a stereoselective binding of -D -Ala peptides have been retained from solution into the gas phase [1]. Thus, in gas phase vancomycin interacts strongly and specifically with Ac_2-L -Lys-D -Ala-D -Ala whereas Ac_2-D -Lys-L -Ala-L -Ala is only very loosely bound. Surprisingly, there is no difference between the destruction cross sections of the specific and the nonspecific complex. This indicates that there is no difference in the compactness of their gas phase structures. It is, thus, possible for the nonspecific peptide to adopt a

conformation which allows the formation of a rather compact complex. But the intermolecular electrostatic binding energy still differs in the complexes since the strength of a hydrogen bond depends critically on the exact geometry of the hydrogen bond donor and acceptor.

Furthermore, we have investigated the effect of changes in the binding pocket of vancomycin on the gas phase stability of its noncovalent complexes. The dissociation efficiency curves clearly show that Ac_2-L -Lys-D -Ala-D -Ala forms significantly weaker hydrogen bonds with CDP-1 than with vancomycin. Removal of chlorine on vancomycin slightly destabilizes the interaction with Ac_2-L -Lys-D -Ala-D -Ala. This behavior correlates well with known solution binding affinity.

References

[1] T.J.D. Jørgensen, D. Delforge, J. Remacle, G. Bojesen, P. Roepstorff, Int. J. Mass Spectrom. Ion Processes 188 (1999) 63.
[2] H.R. Perkins, Biochem J. 111 (1969) 195.
[3] C.J. Coulson, Molecular Mechanisms of Drug Action, Taylor & Francis, London, 1994.
[4] D.H. Williams, B. Bardsley, Angew. Chem. Int. Ed. 38 (1999) 1172.
[5] M. Nieto, H.R. Perkins, Biochem. J. 123 (1971) 789.
[6] T.J.D. Jørgensen, P. Roepstorff, A.J.R. Heck, Anal. Chem. 70 (1998) 4427.
[7] T.J.D. Jørgensen, P. Roepstorff, T. Staroske, D.H. Williams, A.J.R. Heck, J. Chem. Soc., Perkin Trans. 2 (1999) 1859.
[8] T. Staroske, D.P. O'Brien, T.J.D. Jørgensen, P. Roepstorff, D.H. Williams, A.J.R. Heck, Chem. Eur. J. 6 (2000) 504.
[9] T.J.D. Jørgensen, J.U. Andersen, P. Hvelplund, M. Sørensen, Int. J. Mass Spectrom. 207 (2001) 31.
[10] P.H. Dawson, J.B. French, J.A. Buckley, D.J. Douglas, D. Simmons, Org. Mass Spectrom. 17 (1982) 212.
[11] P.H. Dawson, J.E. Fulford, Int. J. Mass Spectrom. Ion Phys. 42 (1982) 195.
[12] D. Schröder, C.A. Schalley, J.N. Harvey, H. Schwarz, Int. J. Mass Spectrom. 185–187 (1999) 25.
[13] N. Potier, P. Barth, D. Tritsch, J.F. Biellmann, A. Vandorsselaer, Eur. J. Biochem. 243 (1997) 274.
[14] K.A. Sannes-Lowery, P. Hu, D.P. Mack, H. Mei, J.A. Loo, Anal. Chem. 69 (1997) 5130.
[15] P.D. Schnier, J.S. Klassen, E.F. Strittmatter, E.R. Williams, J. Am. Chem. Soc. 120 (1998) 9605.
[16] A. van der Kerk-van Hoof, A.J.R. Heck, J. Mass Spectrom. 34 (1999) 813.
[17] V. Gabelica, E. De Pauw, J. Am. Soc. Mass Spectrom. 13 (2002) 91.
[18] S.G. Penn, F. He, C.B. Lebrilla, J. Phys. Chem. B 102 (1998) 9119.
[19] K.X. Wan, T. Shibue, M.L. Gross, J. Am. Soc. Mass Spectrom. 11 (2000) 450.
[20] F. Tureček, J. Mass Spectrom. 33 (1998) 779.
[21] M. Sørensen, J.S. Forster, P. Hvelplund, T.J.D. Jørgensen, S.B. Nielsen, S. Tomita, Chem. Eur. J. 7 (2001) 3214.
[22] M.H. Florencio, D. Despeyroux, K.R. Jennings Org. Mass Spectrom. 29 (1994) 483.
[23] B.A. Grzybowski, A.V. Ishchenko, R.S. DeWitte, G.M. Whitesides, E.I. Shakhnovich, J. Phys. Chem. B 104 (2000) 7293.
[24] M.K. Green, C.B. Lebrilla, Mass Spectrom. Rev. 16 (1997) 53, and references therein.
[25] C.S. Hoaglund-Hyzer, A.E. Counterman, D.E. Clemmer, Chem. Rev. 99 (1999) 3037.
[26] Q. Wu, J. Gao, D. Joseph-McCarthy, G.B. Sigal, J.E. Bruce, G.M. Whitesides, R.D. Smith, J. Am. Chem. Soc. 119 (1997) 1157.
[27] M.R. Mauk, A.G. Mauk, Y.-L. Chen, D.J. Douglas, J. Am. Soc. Mass Spectrom. 13 (2002) 59.
[28] J. Rao, I.J. Colton, G.M. Whitesides, J. Am. Chem. Soc. 119 (1997) 9336.
[29] M.P. Williamson, D.H. Williams, S.J. Hammond, Tetrahedron lett. 40 (1984) 569.
[30] C.M. Harris, R. Kannan, H. Kopecka, T.M. Harris, J. Am. Chem. Soc. 107 (1985) 6652.
[31] D.H. Williams, J.P. Waltho, Biochem. Pharm. 37 (1988) 133.
[32] P.J. Loll, P.H. Axelsen, Annu. Rev. Biophys. Biomol. Struct. 29 (2000) 265.
[33] D.H. Williams, B. Bardsley, Perspect. Drug Discover. 17 (1999) 43.
[34] C.V. Robinson, E.W. Chung, B.B. Kragelund, J. Knudsen, R.T. Aplin, F.M. Poulsen, C.M. Dobson, J. Am. Chem. Soc. 118 (1996) 8646.
[35] V.J. Nesatyy, J. Mass Spectrom. 36 (2001) 950.
[36] H. Rogniaux, A. Vandorsselaer, P. Barth, J.F. Biellmann, J. Barbanton, M. Van Zandt, B. Chevrier, E. Howard, A. Mitschler, N. Potier, L. Urzhumtseva, D. Moras, A. Podjarny, J. Am. Soc. Mass Spectrom. 10 (1999) 635.
[37] Y.T. Li, Y.L. Hsieh, J.D. Henion, T.D. Ocain, G.A. Schiehser, B. Ganem, J. Am. Chem. Soc. 116 (1994) 7487.
[38] X. Cheng, Q. Gao, R.D. Smith, K. Jung, C. Switzer, Chem. Commun. (1996) 747.
[39] V. Gabelica, E. De Pauw, J. Mass Spectrom. 36 (2001) 397.
[40] C.L. Hunter, A.G. Mauk, D.J. Douglas, Biochemistry 36 (1997) 1018.
[41] J. Gao, Q. Wu, J. Carbeck, Q.P. Lei, R.D. Smith, G.M. Whitesides, Biophys. J. 76 (1999) 3253.
[42] E.J. Nettleton, M. Sunde, Z. Lai, J.W. Kelly, C.M. Dobson, C.V. Robinson, J. Mol. Biol. 281 (1998) 553.
[43] A.A. Rostom, J.R.H. Tame, J.E. Ladbury, C.V. Robinson, J. Mol. Biol. 296 (2000) 269.
[44] S.-W. Lee, J.L. Beauchamp, J. Am. Soc. Mass Spectrom. 10 (1999) 347.