Probing the Stability of Proline Cis/Trans Isomers in the Gas Phase with Ultraviolet Photodissociation

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Abstract

Although most peptide bonds in proteins exist in the trans configuration, when cis peptide bonds do occur, they can have major impact on protein structure and function. Rapid identification of cis-peptide bonds is therefore an important task. Peptide bonds containing proline are more likely to adopt the cis configuration because the ring connecting the sidechain and backbone in proline flattens the energetic landscape relative to amino acids with free side chains. Examples of cis proline isomers have been identified in both solution and in the gas phase by a variety of structure-probing methods. Mass spectrometry is an attractive potential method for identifying cis proline due to its speed and sensitivity, however the question remains whether cis/trans proline isomers originating in solution are preserved during ionization and manipulation within a mass spectrometer. Herein, we investigate the gas phase stability of isolated solution-phase cis and trans proline isomers using a synthetic peptide sequence with a Tyr-Pro-Pro motif. A variety of dissociation methods were explored to evaluate their potential to distinguish cis/trans configuration, including collision-induced dissociation, radical-directed dissociation, and photodissociation. Only photodissociation employed in conjunction with extremely gentle electrospray and charge solvation by 18-crown-6 ether was able to distinguish cis/trans isomers for our model peptide, suggesting that any thermal activation during transfer or while in the gas phase leads to isomer scrambling. Furthermore, the necessity for 18-crown-6 suggests that intramolecular charge solvation taking place during electrospray ionization can override cis/trans isomer homogeneity. Overall, the results suggest that solution-phase cis/trans proline isomers are fragile and easily lost during electrospray, requiring careful selection of instrument parameters and consideration of charge solvation to prevent cis/trans scrambling.

Introduction

It has been hypothesized that one of the fundamental steps prior to the evolution of life on Earth was the formation of the peptide bond, allowing the synthesis of proteins from amino acid "alphabet" soup.[1] Peptide bonds are rigid and planar, with local minima in either cis or trans configuration. The vast majority of peptide bonds in proteins adopt the trans configuration, which is energetically favorable relative to cis due to differences in sterics.[2] For non-proline peptide bonds the trans to cis rotational energy barrier is ~83.7 kJ/mol and the potential energy difference between the cis and trans isomers is ~10.5 kJ/mol.[3] The energetics are largely dictated by lower steric clash in the trans configuration. In contrast, the unique five-membered ring inherent to proline causes steric clash in both the cis and trans positions, lowering the transition barrier to ~54.4 kJ/mol and the potential energy difference to only ~2.1 kJ/mol (Scheme 1).[4] This results in Xxx-Pro peptide bonds being more likely to form cis isomers, although Xxx-Pro peptide bonds still favor trans over the cis configuration.[5]

There are specific sequence motifs that have been shown to increase the likelihood of cis isomer formation in proline-containing peptide bonds. A significant percentage of cis-proline bonds are preceded by an aromatic residue, presumably due to stabilizing CH-π interactions between the aromatic residue and the proline.[5,6,7] Out of the three canonical aromatic amino acids, tyrosine precedes cis-proline most frequently in both model peptides and proteins.[2,6] In addition to cis-isomer stabilization from aromatic residues, sequential prolines also affect cis/trans isomerization propensity. In the case of a double-proline motif, four different isomers are possible due to cis/trans isomerization at the Xxx-Pro bond and at the Pro-Pro bond. Kinetic studies of model peptides containing Pro-Pro motifs suggest that sequential prolines significantly slow the rate of isomerization from cis to trans when compared to isomerization in model peptides with a single proline.[2] Combination of both CH-π and proline-proline interactions have been suggested to result in both a greater likelihood of cis-proline formation as well as a

slower rate of isomerization, allowing cis-proline peptide bonds to be investigated with greater ease.[8]

When cis-proline is present, it significantly influences both structure and folding. The relatively slow timescale for proline cis/trans isomerization allows detailed study by NMR for characterization of protein folding events.[9] For example, heteronuclear 2D-NMR has been used to monitor folding of the proline-rich collagen triple helix, revealing that multiple cis/trans proline isomerization events occur during folding.[10] Differential scanning calorimetry coupled with NMR showed slow conversion of trans to cis proline at the Tyr28-Pro29 peptide bond in the homeodomain from the human transcription factor PBX, allowing direct detection of denatured state chemical shifts.[11] Proline cis/trans isomerization has also been shown to play roles in protein function. Peptidyl-prolyl isomerase-accelerated proline cis/trans isomerization in the Crk adaptor acts as a switch between an autoinhibitory function (cis configuration) and an extended and uninhibited structure (trans configuration).[12] Cis-trans proline isomerization has also been established as a mechanism for the opening and closing of a neurotransmitter-gated ion channel.[13] Recently, the importance of cis-trans proline isomerization has been appreciated for modulating antigen-antibody interactions and the development of therapeutic antibodies including the anti-HIV antibody 10E8 that contains a Tyr-Pro-Pro (YPP) motif.[14-18] These examples highlight the biological relevance of proline cis/trans isomerization and establish the importance of utilizing existing techniques including NMR for discovery and characterization of proline cis/trans isomerization.

Scheme 1. Cis/trans isomerization of proline. The energy differences between the cis and trans configurations is ~2.1 kJ/mol.[4]

Gas phase studies of cis/trans proline isomerization have also revealed important insights. Ion mobility combined with mass spectrometry (IM-MS) can be used to separate conformations or derive structural information by comparing collision cross-sections with calculations. Multiple structure populations have been observed with IM-MS for proline-containing peptides, which have been attributed to cis/trans isomers of proline.[19,20,21] Similarly, the transition from polyproline I to polyproline II via the sequential cis-to-trans isomerization of each proline bond has been detailed.[22,23] Ion mobility has also been used in conjunction with ultraviolet photodissociation (UVPD) to probe the differences in higher order gas-phase protein structure arising from proline cis/trans isomerization in ubiquitin.[24] In addition to ion mobility, IRMPD has been used to study gas-phase cis/trans isomers of prolyl bonds in polyproline.[25] IRMPD coupled with ion mobility identified a subpopulation within one of the major bradykinin structures resulting from isomerization to cis-proline at proline 2.[26] Despite the advances made in these studies, the full relationship between solution phase cis/trans isomers and gas phase cis/trans isomers remains an open question.

To examine known solution phase cis/trans proline isomers in the gas phase, the isomers must first be ionized and desolvated without causing structural rearrangement. Electrospray ionization (ESI) is the most commonly used method for attempting such transfers,[27] but the mechanism of ESI is not fully understood despite a great deal of investigation.[28,29,30] It is generally agreed that higher charge states are less likely to preserve solution-phase structure. Greater clarity has been provided by simulations in some cases, such as the chain ejection model proposed by Konermann.[31,32] However, proteins ionized by chain ejection bear little resemblance to their solution-phase structure, and in general, the clearest examples of gas-phase structure or structural transitions are those where the original native protein structure is lost. For example, highly charged ions that have essentially completely unfolded are clearly not native. Undisputed examples of structure retention remain more elusive.

While ESI is normally gentle enough to preserve covalent bonds during transfer into the gas phase, the removal of solvent creates a problem with respect to preservation of solution-phase noncovalent interactions. These interactions are critical to stabilizing native protein structure, and include details such as cis/trans configuration. In theory, mass spectrometric analysis of proline cis/trans isomerization should be readily achievable, but can cis/trans isomers present in solution be preserved during transfer into the gas phase? Although previous studies have indicated that transfer into the gas phase can lead to dramatic rearrangement of protein/peptide structure into non-native conformations, this question has not been fully explored in relation to proline cis/trans isomerization.[33]

In the course of this study, we utilize collisional activation, radical-directed dissociation (RDD), and photodissociation (PD) to assess cis/trans prolyl isomer stability during ESI. A model peptide (WSGYPPEE) derived from a sequence previously known to resolve into multiple liquid chromatography (LC) peaks corresponding to proline isomers is used. The results reveal that cis/trans prolyl isomers are fragile and subject to interconversion during ESI or if subjected to thermal activation. Preservation of these isomers from solution to gas phase for our model peptide requires both gentle ESI parameters and external charge solvation to minimize gas phase rearrangement. Even under these conditions, distinguishing cis/trans isomers is challenging, suggesting they are unlikely to interfere in experiments where not specifically targeted.

Experimental Methods

*Materials***.** Organic solvents and reagents were purchased from Fisher Scientific, Sigma-Aldrich, or Acros Organics and used without further purification. FMOC-protected amino acids and Wang resins were purchased from Anaspec, Inc or Chem-Impex International.

Peptide Synthesis. Peptides were synthesized manually following a FMOC-protected solid-phase peptide synthesis protocol.[34] FMOC-3-iodotyrosine replaced tyrosine during synthesis to

provide a photocleavable radical precursor for RDD experiments. Following synthesis, peptides were stored frozen in 50/50 v/v acetonitrile/water.

Nuclear Magnetic Resonance. 6 mg of modified peptide WSG(I-Y)PPEE was dried fully and resuspended in in 0.104 mM 3-(Trimethylsilyl)-1-propanesulfonic acid (DSS), 50 mM sodium phosphate and pH corrected to pH 2.75 (final peptide concentration of 9.5 mM). The sample was then dried down by speedvac and resuspended in 99.99% D2O twice prior to NMR analysis. Samples were covered with aluminum foil whenever possible to prevent photodegradation.

All spectra were acquired on a Bruker AVANCE III 700 MHz solution NMR Spectrometer equipped with a proton optimized room temperature triple resonance TXI-1H{^{13}C,^{15}N} probehead. The sample was equilibrated for at least 10 minutes at a temperature of 283 K before spectra were recorded. 2D ^{1}H-^{1}H TOCSY (70 ms mixing time, 2048 points along F2 and 1024 points along F1 with 8 scans per increment), ^{1}H-^{1}H ROESY (200 ms mixing time, 2048 points along F2 and 1024 points along F1 with 8 scans per increment) and ^{1}H-^{13}C HSQC at 1% natural abundance of ^{13}C (2048 points along F2 and 512 points along F1 with 96 scans per increment) were recorded and spectra were processed and referenced to the DSS trimethyl silyl resonance using Bruker TopSpin software (version 4.0.8). ^{1}H resonances were assigned using the combination of crosspeaks from the HSQC and spin systems assigned from the TOCSY and ROESY using NMRFAM-SPARKY (version 1.470, powered by SPARKY version 3.190).

Assignment of all resolved peaks are shown in supporting table S1. All assigned chemical shifts were within one standard deviation from the average values reported in the Biological Magnetic Resonance Data Bank.[35] Cis/Trans proline assignments for each isomer were made using the relative intensity of the HA-HA or HD-HA crosspeak,[36] along with the carbon chemical shifts for the assigned prolines.[37] The relative abundance of each isomer was determined by the relative integration of a single resonance associated with each specific isomer. For example, the relative integration of the P5-α-trans-trans resonance was compared directly with P5-α-cis-trans

and P5-α-trans-cis to determine the relative abundance of each population within the sample. The total relative populations were calculated from the P5 Ca, P6 Ca, and Y4 Cb cross-peak intensities.

Analysis. For LCMS, an Agilent 1100 binary pump was used with a Thermo BetaBasic-18 3 µm C18 150 mm x 2.1 mm column interfaced to a Thermo Fisher Scientific LTQ mass spectrometer with a standard ESI source. Samples were eluted using water with 0.1% formic acid as mobile phase A, and acetonitrile with 0.1% formic acid as mobile phase B. A Waters XBridge 3.5 µm C18 4.6 mm x 250 mm column interfaced to a Varian ProStar 320 UV/Vis detector and two ProStar 210 pumps were used to fractionate cis/trans isomers from the iodo-tyrosine peptide variant prior to performing PD and RDD experiments. To minimize interconversion between cis and trans isomers during separation, the column was wrapped with ice packs and the solvents were kept in an ice bath. Separated fractions were prepared as ~20 µM samples in 49.5/49.5/1 (v/v/v) acetonitrile/water/formic acid with a ~1:1 molar ratio of peptide to 18C6 and infused into the LTQ linear ion trap using the standard electrospray ionization source. The LTQ was modified previously with a quartz window to allow 266 nm laser pulses from a Nd:YAG laser to excite the isolated precursor ions and form a radical on the peptide by photodissociation of the carbon—iodine bond. Following photodissociation, the radical product was further isolated and activated by collision-induced dissociation (CID) to promote radical-directed dissociation.

Instrument Parameters. Gentle ESI parameters were obtained by tuning the LTQ source voltages to maximize selective observation of serine octamer without generating any other serine clusters. This 'magic serine octamer' spectrum will only be observed if the ESI conditions are sufficiently gentle,[38] which does not correspond to high signal or analytical sensitivity. The instrument parameters for the gentle ESI tune are as follows: spray voltage 4.4kV, capillary temperature 120.0ºC, capillary voltage 0.00V. The tube lens voltage was varied as follows: -35V, -25V, and +90V (corresponding to decreasingly gentle settings). To evaluate the

effect of more standard ESI conditions, the instrument was tuned to the [M+H] + 18C6 adduct. The resulting parameters were spray voltage 4.4kV, capillary temperature 120.0°C, capillary voltage 23V, and tube lens +190.0V.

R_{isomer} Scores. We employ a method established by Tao *et al*.[39] to distinguish and quantify the differences in fragmentation patterns arising from two different isomers in PD and RDD spectra. R_{isomer} scores are determined by calculating a ratio of ratios R_1 / R_2, where R_1 and R_2 are ratios of two fragment ion intensities for isomers 1 and 2, respectively. Values of R_{isomer} close to one indicate fragmentation patterns that are nearly identical, while spectra with increasing differences in fragmentation give higher R_{isomer} scores. All possible pairs of fragment ions in two sets of spectra are compared, with the pair that gives the highest R_{isomer} score being selected as the pair that distinguishes the isomers the most. We define the minimum R_{isomer} scores that confirm the presence of isomeric forms to be those where differences in both reference peaks are outside two standard deviations.

Results and Discussion

CID and RDD Experiments. In this study, a model synthetic peptide WSGYPPEE (based on the 10E8 antibody) is used to study the retention of cis/trans proline during transfer into the gas phase by ESI. Isomerization of the Tyr-Pro and Pro-Pro bonds can yield up to four possible isomers (trans-trans, cis-trans, trans-cis, and cis-cis). When examined in electrospray-like solutions, three isomers are observed by NMR that correspond to WSG(iodo-Y)transPtransPGEE, WSG(iodo-Y)cisPtransPGEE, and WSG(iodo-Y)transPcisPGEE configurations, with relative intensities of 59.49 ± 5.6%, 33.54 ± 2.9% and 6.97 ± 2.8%, respectively (Figure S1). The lack of any signal observed for the WSG(iodo-Y)cisPcisPGEE is consistent with the low propensity of peptides to adopt the higher energy cis-cis isomer.2

LCMS analysis of the same peptide (WSGYPPEE) produces a chromatogram with two prominent peaks that derive from the same m/z (Figure 1a). The relative abundances of the two

isomers as calculated from peak areas are ~43% and ~57% respectively, indicating elution of the cis-trans isomer first followed by the trans-trans isomer. Collisional activation of the two isomers produces virtually identical fragmentation patterns (Figure 1b and 1c) that are incapable of distinguishing the two isomers. This failure may be in part due to the abundant $y4^+$ ion that dominates both spectra. It is produced by the proline effect that favors CID cleavage N-terminal to proline.[40] It is also likely that the thermal energy added for CID is sufficient to completely scramble cis/trans isomer configuration.

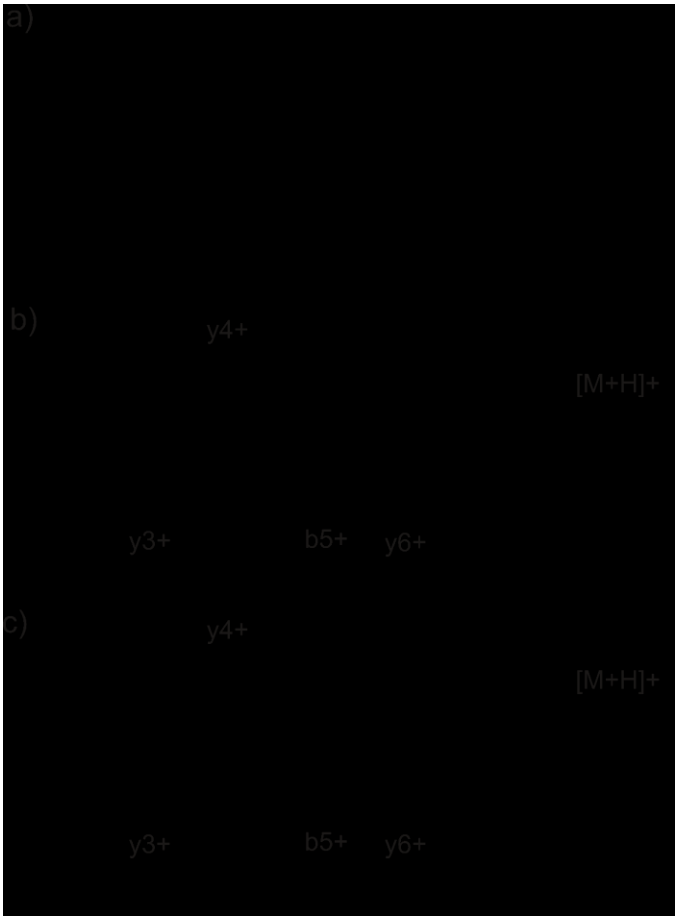

Figure 1. (a) C18 separation on ice of the synthetic peptide WSGYPPEE resulting in separation of the cis-trans and trans-trans isomers at retention time 14.4 minutes and 16 minutes, respectively. **(b)** CID spectrum of the cis-trans isomer eluting at 14.4 minutes and **(c)** CID spectrum for the trans-trans isomer eluting at 16 minutes. The CID spectra are indistinguishable.

Unfortunately, the proline effect may always be a potential problem for peptides that contain proline (and are therefore candidates for proline cis/trans isomerization). Furthermore, CID is not very sensitive to structure in general. However, we have demonstrated previously that radical-directed dissociation (RDD) can identify covalently constrained stereoisomers based on differences in fragmentation.[41,42] This approach can even successfully discriminate challenging isomers such as those derived from aspartic acid and glutamic acid.[43,44] RDD is initiated by photodissociation of a carbon–iodine bond, allowing for selective creation of a radical following activation with ultraviolet light. 3-iodotyrosine was incorporated into our model peptide to facilitate RDD experiments. To establish whether modification of the tyrosine affected the propensity of the YP bond to form cis isomers, we attempted to separate isomers from the modified sequence with LC. Results show that WSG(iodo-Y)PPEE separates into two prominent peaks (Figure 2a), corresponding to cis-trans and trans-trans. To avoid the possibility that proline cis/trans isomers might be scrambled during transit into the gas phase, gentle ESI parameters were employed. Since ESI conditions are more difficult to precisely control when the instrument is operated in LCMS mode, experiments were conducted by direct infusion. The peptide was separated into the two isomers via RP-HPLC with a C18 column while the solvents and column were chilled with an ice bath and cold packs, respectively. The two fractions corresponding to the separated isomer populations were then analyzed promptly using soft ESI tune conditions. The resulting RDD spectra are shown in Figures 2b and 2c. Despite the use of gentle ESI parameters, the data are still quite similar and yield an R_{isomer} score of 1.3. The exact identity of the C-terminal fragment used in the R_{isomer} score is unknown, but further CID on this ion results in an abundant proline effect y_4^+ fragment corresponding to PPEE from the original peptide. Graphing the intensities of the c-terminal and -129W fragments relative to $[M^{\bullet}\text{-NH3+H}]^+$ and representing the standard deviation of the mean with error bars further shows that these fragmentation patterns are not significantly different in intensity (Figure 2d).

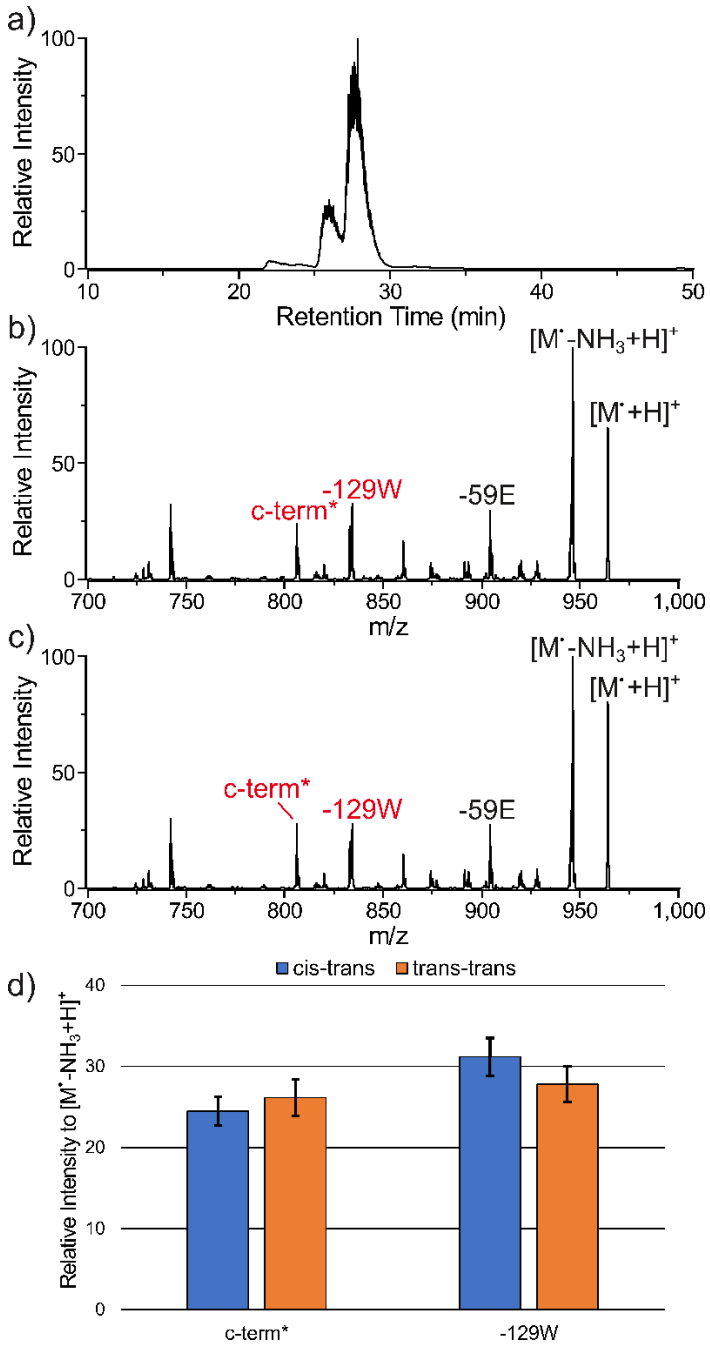

Figure 2. (a) C18 RP-HPLC separation on ice of the synthetic peptide WSG(3-iodo-Y)PPEE. Peaks corresponding to cis-trans and trans-trans isomers at ~25 minutes and ~27.5 minutes were collected on ice and promptly analyzed by RDD to yield the spectra in **(b)** and **(c)**. An R_{isomer} score of 1.3 was obtained using the -129W* and C-terminal fragments shown in red. For this data, a threshold R_{isomer} score of 1.9 would be required to confirm the presence of isomers.

(d) Representation of the relative intensity of the two fragments with the standard deviation of the mean represented by error bars. Inclusion of error bars shows overlap between the -129W intensities and near overlap between the c-terminal RDD fragment. *Side chain losses here and throughout are represented by the shorthand notation corresponding to mass loss and amino acid.[42]

18C6 Photodissociation Experiments. Given that RDD is a structurally sensitive technique that is able to differentiate peptide diastereomers based on L/D isomerization, RDD should have been able to produce differences in fragmentation patterns if the proline cis/trans isomers were able to survive ESI without scrambling and radical migration occurred prior to scrambling once in the gas phase. To further dissect out these possibilities, experiments were conducted with the addition of 18-crown-6 (18C6), which has been shown to solvate charges and aid in the preservation of native-like structure during ESI.[45,46] Using the same source conditions as in the previous protocol with the addition of 18C6 to the isolated fractions prior to direct infusion yielded the PD spectra shown in Figures 3a and 3b. One of the primary losses corresponds to the loss of iodine, as expected. However, further collisional activation of the radical adduct $[M^{\bullet}+18C6+H]^{+}$ to obtain RDD data resulted in spectra that were once again very similar to each other (Figure S2). It is possible that the collisional activation step in RDD enables cis/trans isomer scrambling prior to radical migration, reducing structural sensitivity.

However, two peaks $[M-NH_3+H]^{+}$ and $[M-CO_2+H]^{+}$ correspond to losses from the precursor peptide without loss of iodine. Comparison of these peaks yields a higher R_{isomer} score of 3.5, which is consistent with different isomeric forms. The loss of ammonia and CO_2 are likely due to direct dissociation,[47] meaning that these losses occur prior to any heating. The 18C6 adducts would then be lost afterwards following internal conversion of residual photon energy.[48] Collisional activation of the protonated peptide is dominated by the proline effect, and yields a spectrum similar to that shown in Figure 1 for the non-iodinated peptide for which losses of CO_2

and ammonia are not notable, further suggesting these losses are uniquely attributable to UVPD.

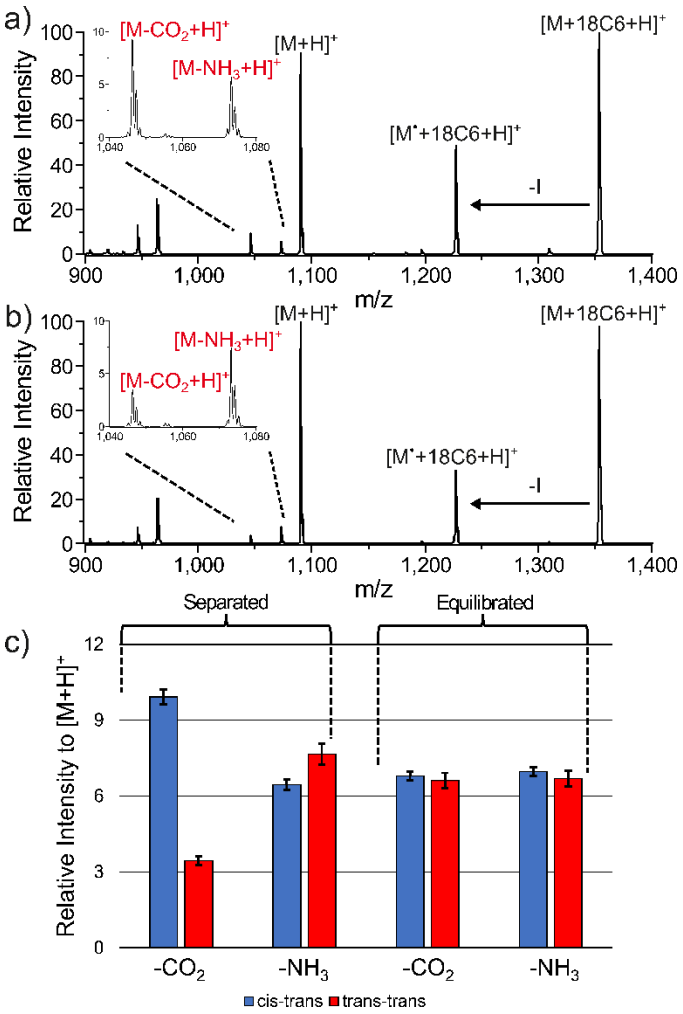

Figure 3. PD spectra resulting from photodissociation of the $[M+18C6+H]^+$ adduct for the cis-trans isomer **(a)** and the trans-trans isomer **(b)**. $[M-NH_3+H]^+$ and $[M-CO_2+H]^+$ are the two fragments that differ the most between the two spectra and are responsible for the R_{isomer} score of 3.5 (relative to minimum threshold of 1.3). **(c)** Bar plot of the intensity for each of these two fragments normalized to the $[M+H]^+$ ion intensity. Error bars represent the standard deviation of the mean, showing significant differences directly after separation, but virtually no difference after equilibration and repetition of the experiments.

To confirm that the differences in intensity noted in Figure 3a and 3b derive from cis or trans configuration, the two fractions were equilibrated at room temperature for 12 hours to allow for cis/trans interconversion. Repetition of the experiments with 18C6 and the same ESI parameters used prior resulted in PD spectra with a much lower R_{isomer} score of 1.3 (see Fig. S3), confirming that the differences observed immediately following separation stem from the cis-trans/trans-trans configurations. The change in behavior is quantitatively illustrated in Figure 3c, where the average intensity and error are plotted for both experiments.

ESI Parameters and Preservation of Cis/Trans Configuration. Over the course of many past experiments, we have noted that the tube lens voltage has the largest influence on the gentleness of the ESI in our instrument. The results of variation of the tube lens voltage from -35, -25, +90 and +190V during collection of PD data with 18-crown-6 are summarized in Figure 4, which show that the losses of CO_2 and NH_3 are most significantly different at more negative tube lens voltages and progressively become less significant as the tube lens voltage increases. Calculating R_{isomer} scores corresponding to these spectra confirms the loss of distinct isomer populations, with the R_{isomer} scores decreasing from 3.5 to 1.6 as represented by the red line in Figure 4. Only the harshness of the ESI conditions are being changed in this series of results, which confirm that activation in the source region can lead to scrambling of cis/trans isomers.

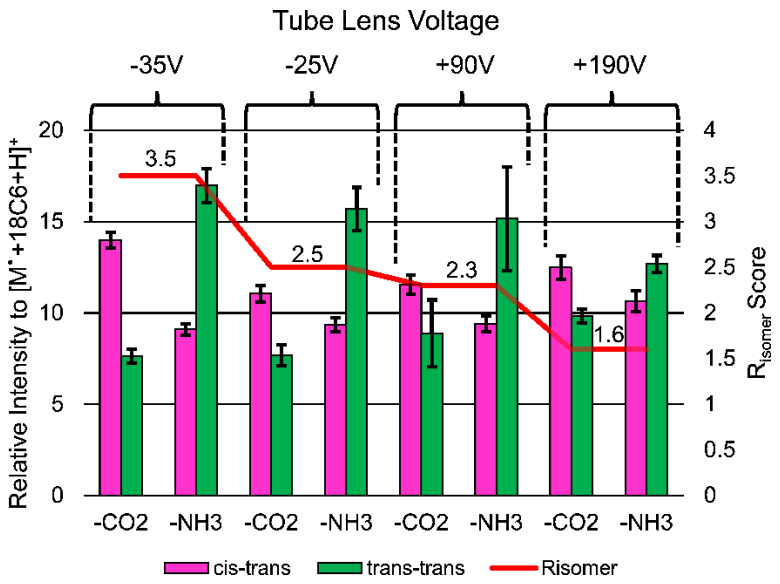

Figure 4. -CO_2 and -NH_3 intensities normalized to $[M^{\bullet}+18C6+H]^+$. Error bars represent the standard deviation of the mean. The cis-trans and trans-trans fractions are represented by the pink and green bars, respectively. The change in R_{isomer} score as determined from the -CO_2 and -NH_3 losses in each pair of fractions is represented by the transparent red line. The fragments gradually become more similar in intensity as tube lens voltage is increased, yielding a corresponding decrease in R_{isomer} score.

When the identical experiment is conducted without addition of 18C6, no significant difference is detected in the losses of CO_2 and NH_3 (see Fig S4). Overall, our data reveal that a combination of charge solvation and gentle ESI conditions are necessary to preserve proline cis/trans isomers during transfer into the gas phase. Use of either of these measures alone is not enough to preserve the isomeric forms. These results suggest that the forces driving charge solvation in the gas phase are sufficient to cause rearrangement of peptide structure and overcome cis/trans isomerization barriers for proline. Consideration of the relevant energetics related to charge solvation is consistent with this idea. For example, estimates of the gas-phase binding energy of 18C6 to a protonated primary amine are in the range of 150-224 kJ/mol.[49,50] Although 18C6 is ideal for solvating primary amines and solvation energies derived from more

ad hoc intramolecular interactions within a peptide may be lower in magnitude, it is still easily conceivable that such solvation energies could exceed the 54.4 kJ/mol[4] required for proline to overcome the barrier between trans/cis configuration. Given that the peptides studied herein are slow to isomerize, it is likely that other sequences would be equally or more difficult to preserve through the process of ESI. Indeed, the relevant energetics suggest that it may be possible for non-proline peptide bonds to be scrambled during ESI due to molecular rearrangement driven by charge solvation.

Conclusion

Our analysis of the WSGYPPEE synthetic peptide and an iodo-tyrosine variant highlight the difficulties associated with transfer of solution-phase cis/trans proline isomers into the gas phase for analysis. A combination of soft ESI conditions and charge solvation, for which we found 18C6 to be suitable, are required to enable transfer with high fidelity. It is also clear that collisional activation once in the gas phase can also facilitate cis/trans isomer scrambling. Although this suggests that future identification of cis/trans prolyl isomers by simple fragmentation-based experiments will be challenging, it also implies that other experiments examining stereoisomers or constitutional isomers under typical MS conditions are unlikely to be complicated by interference from cis/trans proline isomers. Furthermore, these results make it clear that intramolecular charge solvation in the gas phase is a powerful force that can easily overcome some structural preferences that may exist in solution. Due to their highly labile nature, the application of gas-phase results to cis-trans prolyl isomers should probably be supplemented by experiments directly examining the same systems in solution.

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Supporting Information. 2D ^{1}H-^{13}C HSQC NMR spectra of WSG(i-Y)PPEE (Figure S1); RDD spectra of separated cis-trans and trans-trans isomers (Figure S2); Photodissociation spectra of $[M+H+18C6]^{+}$ for cis-trans and trans-trans isomers following room temperature equilibration (Figure S3); Photodissociation spectra of $[M+H]^{+}$ following separation of cis-trans and trans-trans isomers (Figure S4); NMR assignments (Table S1).

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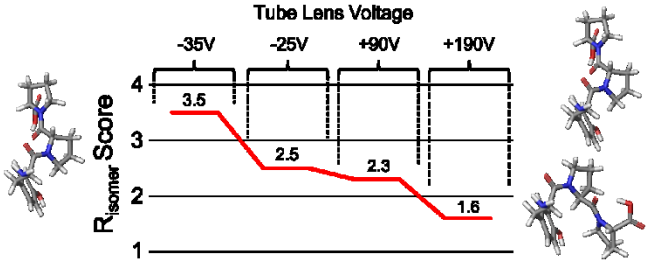
TOC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