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Hydrogen/deuterium exchange of nucleoside analogs in a quadrupole ion trap mass spectrometer

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Abstract

Hydrogen/deuterium (H/D) exchange reactions of protonated nucleoside analogs, including several antibiotics, are reported. Protonated nucleoside analogs contain a wide variety of labile hydrogens and undergo a different number of exchanges when reacted with the reagents ND_3 and CH_3OD. All of the protonated nucleoside analogs, except for tubercidin and cytidine, exchanged all of their labile hydrogens plus the protonating hydrogen when reacted with ND_3. The protonated analogs underwent a more selective exchange with CH_3OD, and only zidovudine exchanged all of its labile hydrogens plus the protonating hydrogen. The results indicate that H/D exchange involves both rings of the nucleoside analogs despite fairly large differences in basicities of specific sites in the two rings, suggesting that the protonating hydrogen can remain mobile or that hydrogen-bond formation between the deuterated reagent and the analyte may occur across the two rings. (Int J Mass Spectrom 190/191 (1999) 161–170) © 1999 Elsevier Science B.V.

Keywords: Hydrogen/deuterium exchange; Quadrupole ion trap; Nucleoside

1. Introduction

Gas-phase hydrogen–deuterium (H/D) exchange reactions studied in a mass spectrometer are becoming a widespread tool for structure elucidation, isomer distinction, and counting the number of labile hydrogens in complex molecules [1–17]. The H/D exchange method in the gas phase typically involves the reaction of a protonated or multiprotonated molecule with a deuterated reagent, such as CH_3OD or ND_3; a process that causes exchange of some of the hydrogens attached to heteroatoms of the analyte ion with deuteriums from the reagent [3]. Although the exact mechanism of H/D exchange is still controversial, it is known that the outcome of the H/D exchange reaction depends on the difference in gas-phase basicity between the reagent and analyte [1] and that the formation of hydrogen bonds between the reagent and analyte plays a dominant role [1]. Various mechanisms of H/D exchange have been proposed [10], along with corresponding potential energy diagrams to show the energetics of the reaction intermediates. The use of H/D exchange reactions has become increasingly popular in the arena of characterization of large biological molecules [4–9] because the H/D exchange process provides complementary information to that obtained by traditional activation/dissociation methods. For example, H/D exchange reactions

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Dedicated to J.F.J. Todd and R.E. March in recognition of their original contributions to quadrupole ion trap mass spectrometry.

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have been used to probe the conformations of proteins in the gas phase [10–16] because the extent of H/D exchange varies for denatured versus active proteins due to the differences in accessibility of hydrogens of the protein. The promise of H/D exchange reactions for providing important information about large molecules has also extended to studies incorporating ion-mobility measurements [17].

In the present investigation, we compare the H/D exchange reactions for a series of protonated nucleoside analogs, including several antibiotics, with the aim of evaluating whether H/D exchange reactions give information that may be used to differentiate structurally similar analogs, such as how small structural differences may affect the extent of H/D exchange. Nucleoside analogs were targeted for several reasons. Nucleoside antibiotics are biopharmaceuticals that mimic the behavior of compounds that occur naturally in the body; this feature makes them excellent candidates for chemotherapeutic agents [18]. For example, they are known to function as inhibitors of viral replication [18]. They may possess an array of substituents that influence or participate in H/D exchange via hydrogen-bond formation. The nucleoside analogs in the present study are composed of two discrete rings (one a tetrahydrofuran ring, one a heterocyclic base) that have different types of active hydrogens and may be modeled separately. Nucleoside antibiotics have been the target of numerous mass spectrometric investigations [19–21], but their H/D exchange reactions have not been studied. However, H/D exchange reactions of seven isomers of methyl guanine, an important analog of the well-known heterocyclic base guanine, have been studied in detail in an FTICR mass spectrometer [22]. It was discovered that the first H/D exchange reaction occurred at different reaction rates for each of seven isomers, and different net numbers of hydrogens were exchanged.

In the present study, the extent of H/D exchange is compared for five protonated nucleoside analogs, four heterocyclic bases, and three other model compounds by using both ND_3 and CH_3OD as reagents (Fig. 1). In general, ND_3 is a less selective reagent because of its greater gas-phase basicity relative to that of CH_3OD [1]. The gas-phase basicity of ammonia is

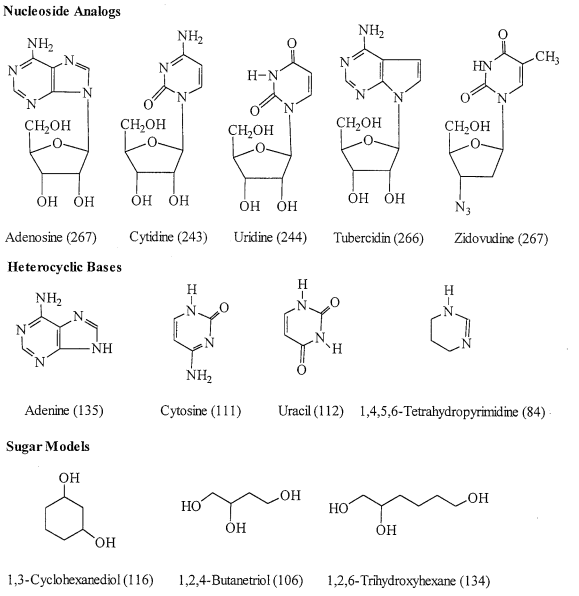


Fig. 1. Structures of nucleoside analogs, heterocyclic bases, and sugar models.

195.8 kcal/mol, whereas the gas-phase basicity of methanol is 173.2 kcal/mol [23]. In comparison, the gas-phase basicities of typical monosaccharides and typical heterocyclic bases range from 196.0 to 201.0 [23] and 201.2 to 219.4 kcal/mol [23], respectively. Thus, the nucleoside portion of the nucleoside analogs contains the more basic regions of these molecules.

2. Experimental

A Finnigan MAT quadrupole ion trap mass spectrometer was used to study the hydrogen–deuterium exchange processes. The standard reagent gas pressure used ranged from 1.0×10^{-5} to 2.0×10^{-5} Torr (1 Torr = 133.3 Pa) and was measured by an ionization gauge positioned in the vacuum chamber. The reagent gas, either methanol-D or ammonia-D_3, was introduced into the chamber by a leak valve. All of the nucleoside analogs and model compounds were introduced by a heated solids probe to nominally $(1–2) \times 10^{-6}$ Torr.

After the nucleoside analogs and model compounds were introduced into the trap, they were

ionized with a short electron ionization pulse and allowed to undergo protonation via self-chemical ionization during a 10 ms period. The deuterated reagent gas was then allowed to react with the compounds. Different reaction times (0−400 ms) were used to vary the degree of H/D exchange that took place. A few reactions involved isolation of the $(M + H)^+$ ion by using the appropriate application of dc and rf voltages, and then allowing it to undergo reactions with the deuterated reagent gas. The mass-selective instability mode was used to eject the fragment ions from the trap on to an electron multiplier for detection.

Collisionally activated dissociation (CAD) experiments were performed after the completion of the H/D exchange. CAD was accomplished through activating the isolated ion by applying an ac voltage of 500 $mV_{p\text{-}p}$ across the end caps at a q_z value between 0.3 and 0.4. CAD was carried out under multiple collision conditions with a background helium pressure of about 1 mTorr. Activation times ranged from 10 to 50 ms.

3. Materials

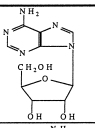
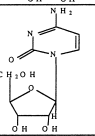
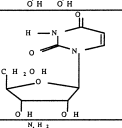
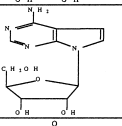
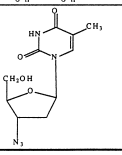
Adenine, adenosine, cytosine, tubercidin, uracil, and zidovudine were obtained from Sigma Chemical (St. Louis, MO, USA), cytidine, 1,4,5,6-tetrahydropyrimidine, and uridine from Aldrich Chemical (Milwaukee, WI, USA), methanol-D from Cambridge Isotopes Laboratories, and ammonia-D_3 from Isotec Inc. (Miamisburg, Ohio, USA). The model compounds 1,2,6-trihydroxyhexane,1,2,4-butanetriol, and 1,3-cyclohexanediol were obtained from Sigma-Aldrich (Milwaukee, WI, USA). All compounds and reagents were used as received.

4. Results and discussion

4.1. H/D exchange reactions of nucleoside analogs

The results of the H/D exchange experiments with the protonated nucleoside analogs are summarized in Tables 1 and 2. Table 1 summarizes the maximum number of hydrogens exchanged for deuteriums when

Table 1
Maximum H/D exchange for the protonated nucleoside analogs[a]

Compound	Structure	Labile H's[b]	# of H's exchanged CH_3OD	# of H's exchanged ND_3
adenosine		5	4	6
cytidine		5	5	5
uridine		4	3	5
tubercidin		5	4	5
zidovudine		2	3	3

[a] Accounts for maximum number of deuteriums incorporated at >5% level.
[b] Does not include the proton attached during ionization.

CH_3OD versus ND_3 is used as the reagent gas. Examples of the H/D exchange spectra are shown in Fig. 2 for protonated uridine using identical reactions conditions with the two different deuterated reagents. The heterocyclic base portion is more basic than the sugar ring [23], so protonation is expected to be thermodynamically more favorable on the heterocyclic base. Upon reaction with CH_3OD, incorporation of one or two deuteriums is most dominant, and the incorporation of three deuteriums is a minor process. In contrast, upon reaction with ND_3, incorporation of up to five deuteriums is observed, with the exchange of second, third, and fourth deuteriums all being dominant processes. Because all five active hydrogens may be exchanged, the H/D exchange process may occur remote from the favored site of protonation (irrespective of the specific site of protonation), suggesting two possibilities. The ionizing proton may

Table 2
Compounds studied and extent of H/D exchange observed with CH_3OD and ND_3 (values rounded off to the nearest 5%)

Sample and reagent		1D	2D	3D	4D	5D	6D
Nucleoside analogs							
Adenosine	CH_3OD	40%	40%	15%	5%	0%	0%
	ND_3	5%	20%	30%	25%	15%	5%
Cytidine	CH_3OD	25%	35%	25%	10%	5%	0%
	ND_3	15%	25%	25%	20%	15%	0%
Tubercidin	CH_3OD	40%	40%	10%	5%	<5%	0%
	ND_3	10%	20%	30%	25%	10%	<5%
Uridine	CH_3OD	65%	25%	10%	0%	0%	0%
	ND_3	10%	25%	30%	25%	10%	0%
Zidovudine	CH_3OD	60%	30%	10%	0%	0%	0%
	ND_3	50%	35%	10%	<5%	0%	0%
Heterocyclic bases							
Adenine	CH_3OD	35%	40%	20%	5%	0%	0%
	ND_3	35%	35%	25%	5%	0%	0%
Cytosine	CH_3OD	60%	30%	10%	0%	0%	0%
	ND_3	25%	45%	20%	5%	0%	0%
Uracil	CH_3OD	75%	20%	<5%	0%	0%	0%
	ND_3	20%	40%	35%	<5%	0%	0%
Model compounds							
1,2,4-Butanetriol	CH_3OD	20%	35%	30%	15%	0%	0%
	ND_3	25%	35%	25%	15%	0%	0%
1,3-Cyclohexanediol	CH_3OD	55%	35%	10%	0%	0%	0%
	ND_3	25%	45%	30%	0%	0%	0%
1,2,6-Trihydroxyhexane	CH_3OD	35%	40%	20%	5%	0%	0%
	ND_3	20%	35%	30%	15%	0%	0%

remain somewhat mobile, shifting between the basic nitrogen and oxygen atoms on both rings via intramolecular hydrogen bonding occurring between different functional groups of the two rings. Proton mobility has been proposed for other protonated basic molecules, such as amino acids and peptides [25–27]. Alternatively, the deuterated reagent may interact most strongly with the proton attached to the nucleoside, but still allow formation of secondary hydrogen bonds with other nitrogen or oxygen donors that could lead to interchange of hydrogen atoms. The gas-phase basicity of the uracil ring is 201.2 kcal/mol, which is greater than that of a typical triol, 196.0 kcal/mol, which serves as a simple model of a sugar. As one scenario, it is possible that a deuterium atom of ND_3 may interact with a hydroxyl oxygen of the saccharide ring via formation of a hydrogen bond while the nitrogen of ND_3 remains hydrogen bonded to the ionizing proton that is attached to the heterocyclic base ring, thus promoting H/D exchange remote from the site of protonation.

For most of the protonated nucleoside analogs, reactions with ND_3 result in exchange of all active hydrogens (including the protonating hydrogen from initial ionization), whereas reactions with CH_3OD result in a lower number of exchanges. The observation that reactions with ND_3 promote a greater extent of H/D exchange agrees with earlier studies that have shown that the higher gas-phase basicity of ND_3 results in less selective H/D exchange with different active hydrogens in a molecule [1]. The basicities of the heterocyclic bases (201.2–219.4 kcal/mol) are closer to the gas-phase basicity of ND_3 (195.8 kcal/mol) than to the gas-phase basicity of CH_3OD (173.2 kcal/mol). As listed in Table 3, protonated cytosine and adenine are the most basic heterocyclic bases.

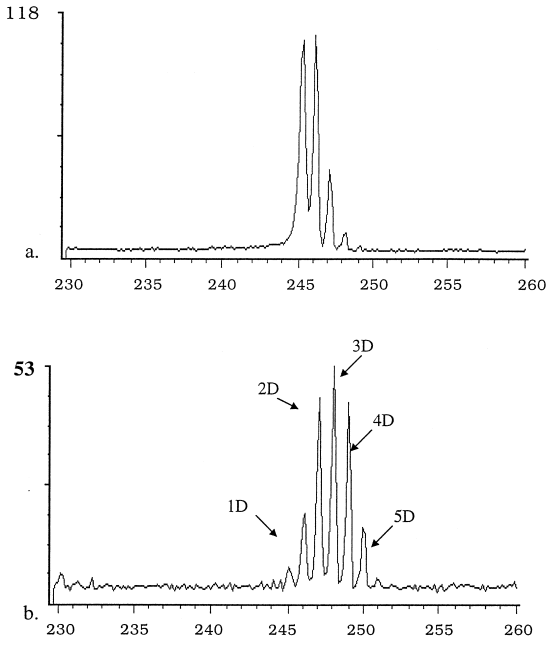
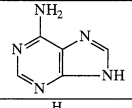
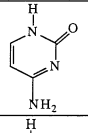
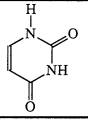


Fig. 2. Hydrogen/deuterium exchange spectra of protonated uridine reacting for 100 ms with (a) CH_3OD and (b) ND_3.

They both exchange all of their labile hydrogens plus the protonating hydrogen when reacting with ND_3. However, when protonated cytosine reacts with CH_3OD, it only undergoes two hydrogen/deuterium exchanges, whereas protonated adenine undergoes the maximum number of exchanges. Due to the lower gas-phase basicity of CH_3OD relative to the basicity of ND_3, it is known that CH_3OD frequently results in less extensive and more selective H/D exchange than that promoted by ND_3 [1,7,10]. Clearly the difference in relative gas-phase basicities of the reagent and the nucleoside analog is not the only factor that influences the H/D exchange reactions because the various nitrogen atoms on the heterocyclic base portion of the analogs are much more basic than CH_3OD, a factor that would tend to inhibit H/D exchange for those compounds. Thus, other factors, such as steric effects and the capability for hydrogen-bond formation, also influence the extent of H/D exchange. The same maximum extent of H/D exchange was noted for the reactions of protonated zidovudine, the only analog that lacks two of the hydroxyl groups on the second ring, with ND_3 and CH_3OD. In view of the more extensive H/D exchange of protonated zidovudine relative to the less extensive exchange of protonated cytidine and adenine, this result suggests that the remote hydroxyl groups of protonated cytidine and protonated adenine are the slowest to exchange. With respect to the maximum incorporation of deuterium relative to the number of total labile hydrogens, the extent of H/D exchange with protonated cytidine was the least efficient for exchange reactions with both ND_3 and CH_3OD. The inefficiency of deuterium incorporation is partly because of the lability of cytidine that causes it to decompose rapidly upon desorption and does not allow accurate statistical studies of H/D exchange.

Table 3
Maximum H/D exchange for the protonated heterocyclic bases[a]

Compound (gas-phase basicity)	Structure	Labile H's[b]	# of H's exchanged CH_3OD	# of H's exchanged ND_3
adenine 218.1 kcal/mol		3	4	4
cytosine 219.4 kcal/mol		3	3	4
uracil 201.2 kcal/mol		2	2	3

[a] Accounts for maximum number of deuteriums incorporated at >5% level.

[b] Does not include the proton attached during ionization.

A summary of the distribution of deuterium incorporation is given in Table 2 for reactions with CH_3OD versus ND_3. The product distributions were calculated by adding the integrated peak intensities of all the deuterated products and converting each ion intensity to a percentage of the total ionization. Since it is difficult to precisely control the pressures of the nucleoside analogs and the deuterated reagents, the distributions allow a qualitative comparison of the extent of deuterium incorporation with an emphasis on which numbers of exchanges are most favored. Products observed at less than 5% of the total ion

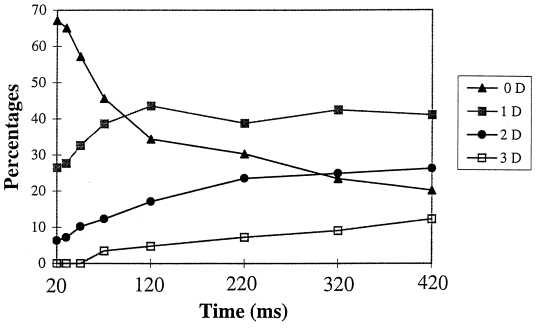
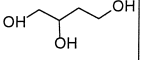
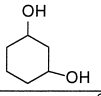
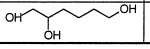


Fig. 3. Time-resolved plot of reactions of protonated uridine with CH_3OD.

current are listed but are somewhat irreproducible from day-to-day. The data in Table 2 represent the results for only a single slice of time and thus give a kinetic snapshot of the H/D exchange reactions. A more detailed kinetic analysis can be obtained by monitoring the H/D exchange reactions as a function of time. An example of this is shown in Fig. 3 for the reactions of protonated uridine with CH_3OD. The dominant changes in the overall product distributions occur in the first 100 ms, and then the incorporation of deuterium continues to occur slowly at longer times. The product containing only one deuterium remains the dominant product at very long reaction times, thus suggesting that the first exchange is always the fastest and generally involves the most labile hydrogen.

Most of the protonated nucleoside analogs undergo overall slow H/D exchange when reacting with CH_3OD: this is signified by the $(M + 1D)^+$ peak being the dominant peak over the more fully exchanged products (Table 2). Protonated cytidine reacts more efficiently with CH_3OD than the other nucleoside analogs, as evidenced by the intensity of the $(M + 2D)^+$ peak being larger than the $(M + 1D)^+$ peak. Protonated tubercidin and adenosine also react somewhat more efficiently with CH_3OD than the other nucleoside analogs, as demonstrated by the intensity of their $(M + 2D)^+$ peak being equal to the $(M + 1D)^+$ peak. Protonated adenosine, tubercidin, and uridine all undergo rapid exchange with ND_3, signaled by their $(M + 3D)^+$ peaks being the most dominant ions. Protonated cytidine undergoes a

Table 4
Maximum H/D exchange for the protonated sugar models[a]

Compound	Structure	Labile H's[b]	# of H's exchanged CH_3OD	# of H's exchanged ND_3
1,2,4-Butanetriol		3	4	4
1,3-Cyclohexanediol		2	3	3
1,2,6-Trihydroxyhexane		3	4	4

[a] Accounts for maximum number of deuteriums incorporated at >5% level.
[b] Does not include the proton attached during ionization.

slower reaction with ND_3 as compared to the other nucleoside analogs, as evidenced by the $(M + 2D)^+$ and the $(M + 3D)^+$ peaks being larger than the $(M + 1D)^+$. Of all the nucleoside analogs protonated zidovudine undergoes the slowest exchange with ND_3 as shown by the dominant $(M + 1D)^+$ peak.

4.2. H/D exchange reactions of models

H/D exchange reactions were undertaken for a series of model compounds that represent the two types of rings of the nucleoside analogs to provide more insight into the extent of H/D incorporation. The results are summarized in Tables 2–4, and an example of the H/D exchange spectra is shown in Fig. 4. Fig. 4 illustrates the extent of H/D exchange for reactions of protonated uracil with CH_3OD and with ND_3. The reactions with CH_3OD result predominantly in incorporation of one deuterium, whereas the reactions with ND_3 leads to incorporation of two or three deuteriums. These spectra confirm that the low basicity of CH_3OD prevents efficient exchange with protonated uracil, which possesses two active hydrogens plus the protonating hydrogen.

As seen in Table 3, all of the protonated heterocyclic bases are able to exchange all of their active hydrogens upon reaction with ND_3, whereas reactions with CH_3OD prove to be somewhat more selective. Based on these results, one would expect that ND_3 could promote exchange of all active hydrogens of the

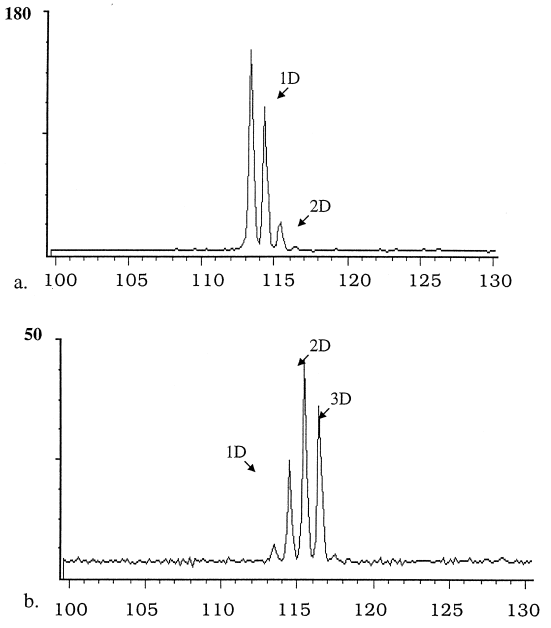
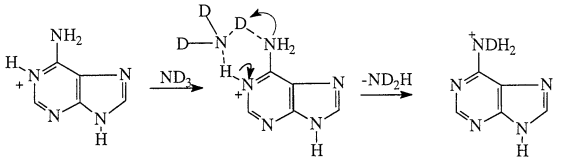


Fig. 4. H/D exchange spectra of protonated uracil reacting for 50 ms with (a) with CH_3OD and (b) ND_3.

heterocyclic base portion of the protonated nucleoside analogs, even when the hydrogens are located at opposite sides of the ring, as is seen in the case of cytosine. The results for reactions with CH_3OD suggest that H/D exchange is only possible when the active hydrogens are close to the site of protonation and/or when a carbonyl group is not present. The carbonyl group may cause several effects that impede H/D exchange. First, the carbonyl group exerts an electron-withdrawing effect on the adjacent nitrogens, thus reducing their basicity. Second, the difference in basicities between the nitrogen atoms and carbonyl oxygen may prevent the formation of the stable hydrogen bonds that are critical for the H/D exchange. For example, the favored site of protonation of adenine is at the nitrogen located adjacent to the primary amino group [24]. However, the protonating hydrogen may remain mobile, as mentioned previously in the discussion of the reactions of the protonated nucleoside analogs [25–27]. In addition, the positions of the other nitrogens facilitate multiple intermolecular hydrogen-bond formation between the deuterated reagent and adenine and allow the relay mechanism of



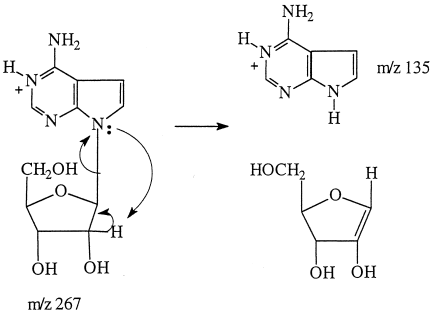
Scheme 1. Possible mechanism of H/D exchange for protonated adenine.

H/D exchange [10] (see Scheme 1). These features promote efficient H/D exchange for all of the labile hydrogens of protonated adenine. On the other hand, upon reactions with CH_3OD complete H/D exchange does not occur for protonated cytosine which has two active hydrogens on the amine functional group which is on the opposite side of the ring as the other active hydrogen. Only three deuteriums are incorporated upon reaction of protonated cytosine with CH_3OD, suggesting that the lone active hydrogen on the far side of the ring is not involved in the H/D exchange reactions. Likewise for protonated uracil, the other model with a carbonyl group in the ring, the maximum incorporation of deuteriums is not observed upon reaction with CH_3OD, presumably because CH_3OD is less basic, forms weaker hydrogen bonds, and is not able to effectively interact with the labile hydrogen on the nitrogen at the opposite side of the ring. The presence of the carbonyl group in the ring seems to be the key feature that quenches complete H/D exchange for cytosine and uracil upon reactions with CH_3OD. Reactions of protonated tetrahydropyrimidine with the two deuterated reagents gives further confirmation that it is the presence of a carbonyl group that reduces the reactivity of protonated cytosine and protonated uracil. Protonated tetrahydropyrimidine has two active hydrogens including the ionizing proton but no carbonyl groups. It undergoes exchange of both active hydrogens upon reaction with CH_3OD or ND_3, thus exhibiting different behavior than the two carbonyl-containing analogs.

Since sugars could not be thermally desorbed sufficiently well for H/D exchange experiments, compounds containing several hydroxyl groups were selected as model compounds. The results of the H/D exchange reactions are shown in Table 4. In every

case, reactions with ND_3 and CH_3OD resulted in the same amount of deuterium incorporation. This result stems from the relatively low gas-phase basicities of the hydroxyl-containing models relative to the gas-phase basicities of the two deuterated reagents, a factor that reduces the overall selectivity of the ND_3 versus CH_3OD reactions [1,7,10]. For protonated 1,3-cyclohexanediol, the number of deuteriums incorporated likewise corresponded to the maximum number (number of labile hydrogens plus the protonating hydrogen) expected, despite the fact that the two hydroxyl groups are positioned further from each other than in the acyclic models. This result shows that 1,3 positioning of the hydroxyl groups still allows interchange of the protonating hydrogen and facilitates H/D exchange for both hydroxyl groups.

The data for these models suggest that the H/D exchange process is especially favorable when the various active hydrogens are attached to functional groups that may allow interaction between the protonated site and the deuterated reagent. This factor may partly explain the less efficient deuterium incorporation for protonated cytidine and tubercidin, neither of which underwent exchange of all possible hydrogens even with ND_3. The orientation of the sugar and heterocyclic rings may prevent the formation of hydrogen bonds that are needed for H/D exchange in protonated tubercidin and cytidine and slow down the rate of H/D exchange such that the maximum extent of H/D exchange is not observable. In addition, it is interesting to note that two of the models, cytosine and uracil, underwent less efficient exchange with CH_3OD than the reactions of their nucleoside counterparts, cytidine and zidovudine, respectively. For the reactions of protonated cytosine and uracil, it was speculated that the presence of the carbonyl groups prevented efficient H/D exchange across the rings. However, for both cytidine and zidovudine, one of the active hydrogens found in the corresponding models of the heterocyclic base portions is replaced by the formation of a nitrogen-carbon bond that links the sugar ring to the heterocyclic base. Therefore, H/D exchange involving this site does not play a role in the reactions of the protonated cytidine or zidovudine,



Scheme 2. Fragmentation of protonated tubercidin.

and thus the slow exchange across the heterocyclic ring noted for the models is no longer an issue.

4.3. Collisionally activated dissociation

To assess the utility of collisionally activated dissociation for probing the sites of deuterium incorporation, CAD spectra of each the various *n*-deuterated ions of protonated tubercidin and adenosine were acquired (i.e. where *n* equals one, two, three, four, or five deuteriums). For example, protonated tubercidin dissociates predominantly by cleavage of the sugar ring, resulting in a protonated heterocyclic base at *m*/*z* 135 (see Scheme 2). The CAD results show that for the fragmentation process illustrated in Scheme 2, the resulting fragment ions contain a variable number of deuteriums. When the precursor ion that incorporates four deuteriums is activated, fragment ions containing one, two, or three deuteriums are formed, meaning that three, two, or one deuteriums, respectively, end up attached to the neutral sugar portion that is eliminated. This variation in the number of deuteriums in the fragment ions suggests two possibilities. First, the sequence of deuterium exchange of the protonated analog may not be uniform during formation of the *n*-deuterated ions, leading to some ions in which the deuteriums attach first at the sugar portion and some in which the deuteriums attach first at the heterocyclic base, leading to a variety of deuterated isomeric structures. Alternatively, extensive scrambling of the hydrogens and deuteriums may occur during activation. These results indicate that the CAD mass spectra yield nonspecific information about the

locations of the deuteriums in the protonated molecules and the sequence of H/D exchange, and thus the CAD experiments were not pursued further.

5. Conclusions

H/D exchange of protonated nucleoside analogs with ND_3 and CH_3OD has been studied in a quadrupole ion trap mass spectrometer. ND_3, being the more basic reagent, was found to exchange all labile hydrogens plus the protonating hydrogen with the analogs, except with tubercidin and cytidine. When protonated tubercidin reacted with ND_3, five of its six (including the protonating hydrogen) active hydrogens were exchanged. Protonated cytidine also exchanged all but one of its labile hydrogens, once again including the protonating hydrogen attached during ionization. For the exchange reactions with CH_3OD and the analogs, in no case was there full H/D exchange, except with protonated zidovudine. Protonated zidovudine reacted with CH_3OD and exchanged all of its labile hydrogens, plus the protonating hydrogen attached during ionization. When CH_3OD is used, a more selective exchange occurred. These results indicate that H/D exchange occurs quite efficiently and involves both rings of the nucleoside analogs despite fairly large differences in basicities of specific sites in the two rings, suggesting that the protonating hydrogen may remain somewhat mobile or facile hydrogen-bond formation between the deuterated reagent and the analog may occur across the two rings. The overall extent of H/D exchange is thus influenced by small structural differences within the class of compounds and could be useful for distinguishing certain types of isomers.

The collisionally activated dissociation data showed that there was variation in the number of deuteriums incorporated into the fragment ions, thus indicating that either the sequence of H/D exchange involving the active sites on the two rings varied during formation of the deuterated ions or that H/D scrambling occurred during activation. These results verified that CAD experiments would give only ambiguous information about the sequence of H/D exchange.

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