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The HDX approach to evidence the stepwise character of controlled enantioselective reduction of copper^{II} complexes with polar amino acids

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OBJECTIVE

Interpretation of the mechanism of the enantioselective reduction observed during fast consecutive dissociations of quaternary complex $[(^L/D\text{Pgly}+^L\text{Pro}+^L\text{AA-H})+\text{Cu}^{\text{II}}]^+$ and consistent with the weakening of this reaction with AA=Asp and Glu using H/D exchanges

INTRODUCTION

The distinction of chiral amino acids ($^L\text{AA}/^D\text{AA}$) based on competitive $^L\text{AA}_{\text{ref}}$ (or $^L\text{AA}'_{\text{ref}}$) and $^L/D$ AA releases under CID conditions from diastereomeric complexes $[(^L\text{AA}_{\text{ref}}+^L\text{AA}'_{\text{ref}}+^L/D\text{AA-H})+\text{Cu}^{\text{II}}]^+$ formed in electro-spray source was first proposed by Cooks twenty years ago [1]. Later, an enantioselective reduction especially favored from homochiral copper quaternary complexes (CQC) with $^L\text{AA}_{\text{ref}}=\text{L-phenylglycine}$ ($^L\text{Pgly}$) and $^L\text{AA}'_{\text{ref}}$ reagent as e.g. L-proline (^LPro) has been reported. This chiral effect was highlighted by the extent of radical ($^L/D\text{Pgly-H}$) loss from these CQC (Table 1). It has been shown that this reduction was instrument dependent (ion residence duration in the activation zone and the excitation mode, resonant or not; Figure 1). This dependence related to the kinetic shift of $\text{Cu}^{\text{II}} \rightarrow \text{Cu}^{\text{I}}$ reduction (formal loss of (Pgly-H) \cdot radical) shows that its frequency factor is weaker than that of direct Pgly loss. In addition, using double resonance experiment in a ICR cell of FT/MS, it has been shown that the ejection of the furtive $[\text{CQC-CO}_2]^+$ product ion results in a decrease in the abundance of the reduction product ion [2]. This means that the reduction process takes place through CO_2 loss from the Pgly partner promptly followed by the consecutive benzylamine radical release (Figure 2).

Table 1. Comparison of chiral effects (R'_{chiral}) for acidic AA (Asp and Glu) and non-polar AA (Leu and Ile) from CID spectra of the $[(^L/D\text{Pgly}+^L\text{Pro}+^L\text{AA-H})+\text{Cu}^{\text{II}}]^+$ complexes

Pgly	Pro	AA	$r' = I_{\text{Pgly-H}}/I_{\text{Pgly}}$	$R'_{\text{chiral}} = r'/r'_D$
$^L\text{Pgly}$	^LPro	^LAsp	0.14	1.81
$^D\text{Pgly}$	^LPro	^LAsp	0.077	
$^L\text{Pgly}$	^LPro	^LGlu	0.17	2.65
$^D\text{Pgly}$	^LPro	^LGlu	0.064	
$^L\text{Pgly}$	^LPro	^LLeu	3.33	10.09
$^D\text{Pgly}$	^LPro	^LLeu	0.33	
$^L\text{Pgly}$	^LPro	^LIle	2.32	11.60
$^D\text{Pgly}$	^LPro	^LIle	0.20	

In this study, H/D exchange (HDX) approach was applied to investigate the behavior of copper quaternary complexes with $^L/D\text{AA}=\text{L/DGlu}$ deuterated at mobile proton positions (DCQC) towards CID by variation of activation time.

METHODS

The mixtures of $^L/D\text{AA}/\text{Pgly}$ (20 μM) and CuCl_2 (60 μM) were prepared in $\text{MeOH}/\text{H}_2\text{O}$ (50:50) or $\text{D}_2\text{O}/\text{MeOD}$ for HDX in solution. First, the analyses were performed on an LTQ/Orbitrap in the positive ESI mode using direct injection at 15 $\mu\text{L}/\text{min}$. The capillary voltage was set at 3.5 kV and the heat temperature at 100°C. The experiments were performed under resonant (CID in LTQ cell) and non-resonant (HCD in hexapole cell) excitation conditions. Different q_z values and variable excitation time (ActTime) between 10 ms and 3000 ms for CID and 0.1 ms to 3000 ms for HCD were applied for ion selection and excitation. ERMS and sequential MSⁿ experiments were performed on both the labelled and unlabelled CQC precursor ions and product ions. The same conditions were applied (except the limit of ActTime at 1000 ms) when Orbitrap Fusion (Thermo Fischer Scientific) was used (Figure 3).

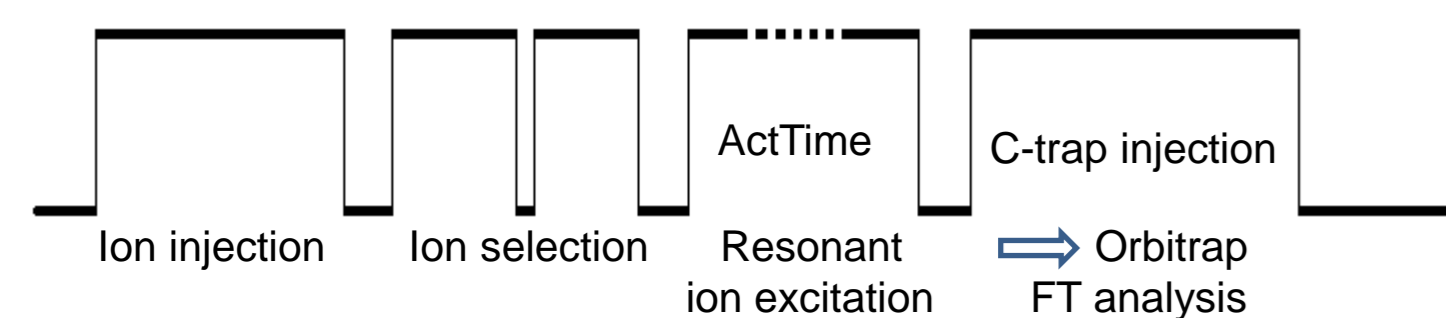


Figure 3. Sequence used for variation of ActTime for both the CID (R) and HCD (NR) experiments

RESULTS

1. Weak enantioselective copper reduction in the case of unlabeled CQC ion under resonant excitation conditions favored by the increase in reaction time without enhancement of chiral effects

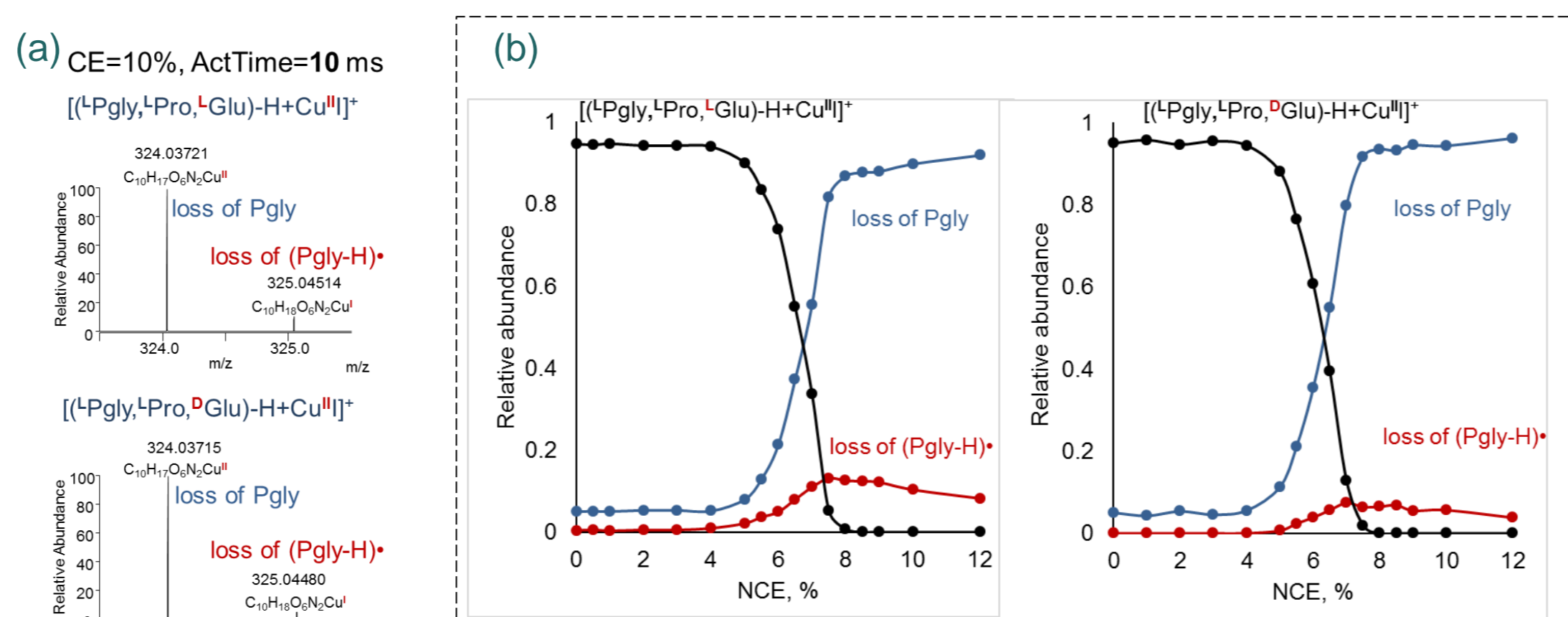


Figure 4. (a) Region of interest of product ion spectra (CID mode, NCE = 10%, ActTime = 10 ms) of homochiral $[(^L\text{Pgly}+^L\text{Pro}+^L\text{AA-H})+\text{Cu}^{\text{II}}]^+$ and heterochiral $[(^L\text{Pgly}+^L\text{Pro}+^D\text{AA-H})+\text{Cu}^{\text{II}}]^+$ CQC ions (m/z 475) and (b) energy resolved CID breakdown curves for diastereomeric CQC ions with the relative abundances of survivor precursor ions and competitive product ions normalized to the total ionic current.

According to Table 1 and Figure 4a, the enantioselective $\text{Cu}^{\text{II}} \rightarrow \text{Cu}^{\text{I}}$ reduction for AA=Glu (or Asp) is much weaker than for non-polar AA (e.g. Leu or Ile). Nevertheless, in a general way, the homo-chiral CQC diastereomeric ions dissociate with larger reduction yield although the direct competitive AA losses are lowered compared to the heterochiral CQC ions (Figure 4b).

The decrease in the reduction yield when all the precursor ion population is consumed (and/or ejected from LTQ) likely means that the average internal energy of dissociating ions is too high. This prevents Cu^{II} reduction (consecutive $(\text{CO}_2+\text{C}_6\text{H}_5\text{-CH}\cdot\text{ND}_2)$ releases) in favor of direct Pgly loss.

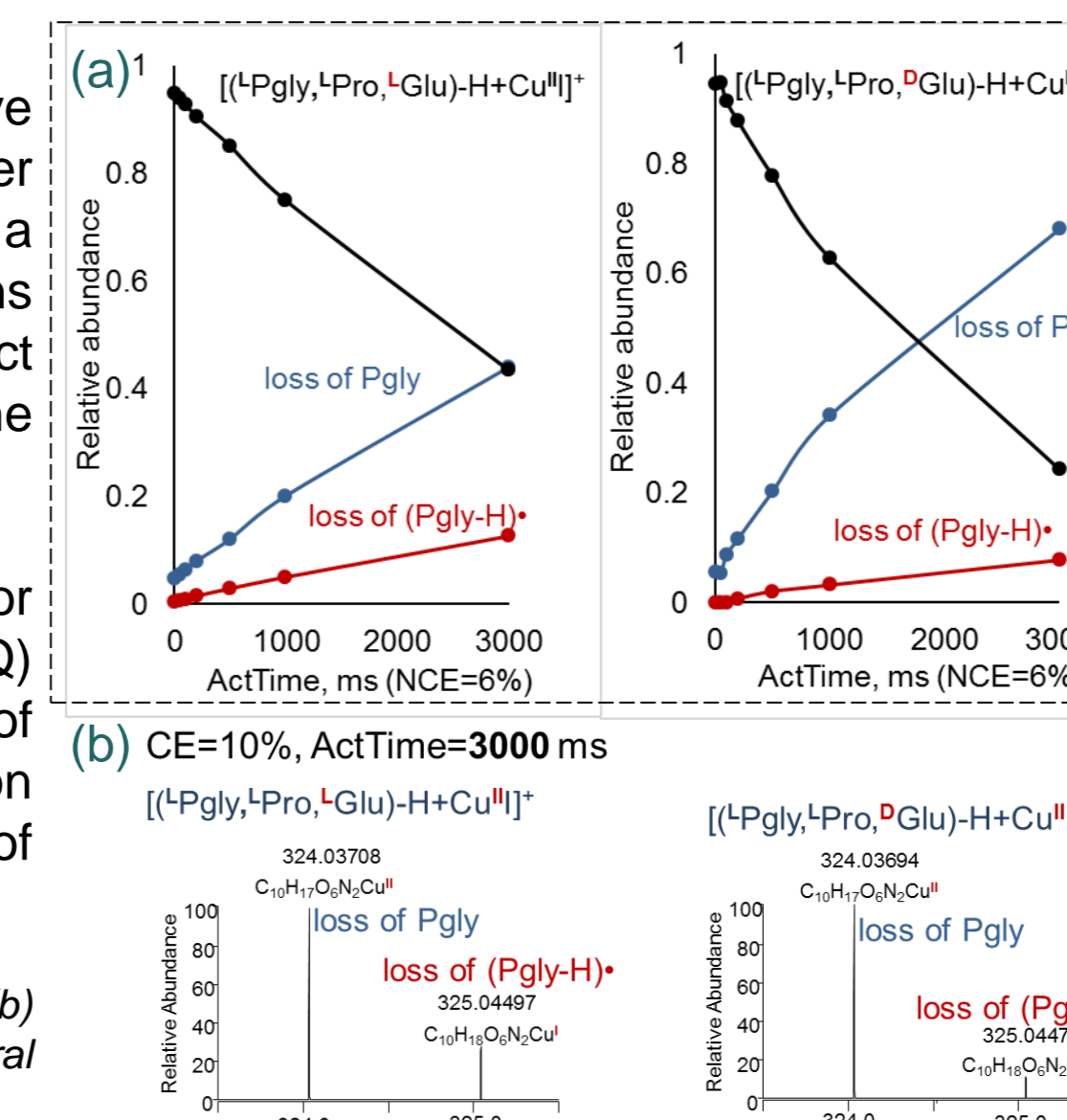


Figure 5. (a) CID duration (ActTime) effect on the reduction yield and (b) region of interest of CID spectra (NCE = 10%) of homo and heterochiral CQC ions (m/z 475) at ActTime = 3000 ms.

Table 2. CID duration (Act Time) effect on the reduction yield (i.e., ratio $r = \frac{[(^L\text{Pro},^L\text{Glu})+\text{Cu}^{\text{I}}]^+}{[(^L\text{Pro},^D\text{Glu})+\text{Cu}^{\text{I}}]^+}$) and ratio $R'_{\text{chiral}} = \frac{r_{\text{Hom}}}{r_{\text{Hetero}}}$

	$\frac{[(^L\text{Pro},^L\text{Glu})+\text{Cu}^{\text{I}}]^+}{[(^L\text{Pro},^D\text{Glu})+\text{Cu}^{\text{I}}]^+}$		$R'_{\text{chiral}} (\pm 0.19)$	
	10ms	3000 ms	10 ms	3000 ms
$^L\text{Pgly},^L\text{Pro},^L\text{Glu}$	0.17 (13)*	0.31 (0.77)*	2.83	2.58
$^L\text{Pgly},^L\text{Pro},^D\text{Glu}$	0.06 (10)*	0.12 (0.44)*		

*These values correspond to the abundance ratios of precursor ion over product ions

By increasing ActTime from 10 to 3000 ms (Figure 5 and Table 2), it appears that:

- the homochiral CQC ion is characterized by a slightly higher stability than the heterochiral one,
- both (Pgly-H) \cdot and Pgly neutral losses are reinforced but the reduction is more favored, however, the R'_{chiral} ratio remains almost constant.

2. Evidence in favor of a stepwise reduction process based on unexpected HDX between AA partners. Origin of the decrease in the reduction reaction yield and its enantioselectivity with acidic AA (Asp and Glu)

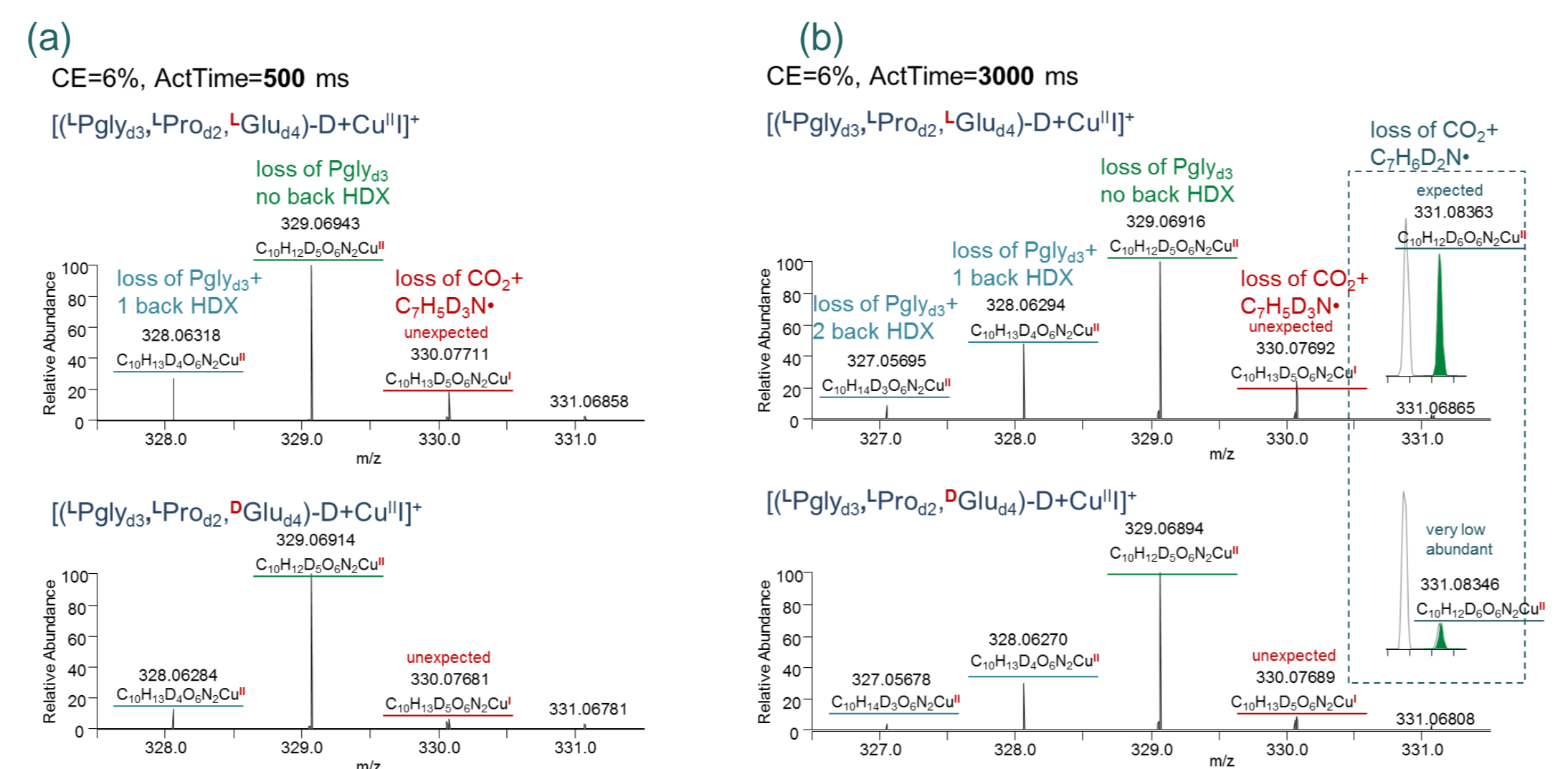
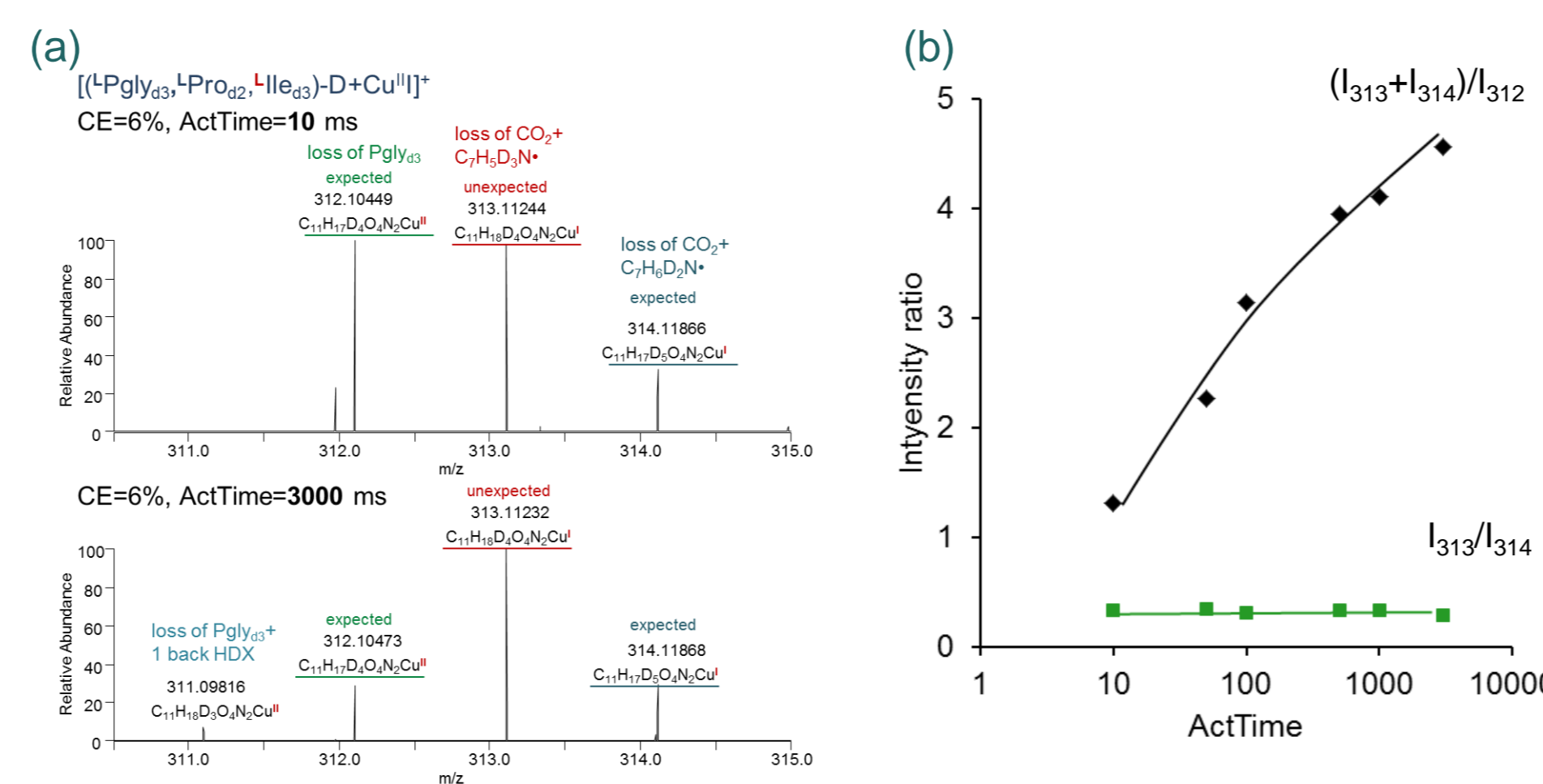


Figure 6. Comparison of the regions of interest of the product ion spectra of homochiral $[(^L\text{Pgly}_{d3}+^L\text{Pro}_{d2}+^L/D\text{Glu}_{d4})-\text{D}+\text{Cu}^{\text{II}}]^+$ and heterochiral $[(^L\text{Pgly}_{d3}+^L\text{Pro}_{d2}+^D\text{Glu}_{d4})-\text{D}+\text{Cu}^{\text{II}}]^+$ CQC ions (m/z 493) activated during (a) 500 ms and (b) 3000 ms in CID mode at NCE = 6%.

The survivor population of completely labeled DCQC $[(^L\text{Pgly}_{d3}+^L\text{Pro}_{d2}+^L/D\text{Glu}_{d4})-\text{D}+\text{Cu}^{\text{II}}]^+$ at m/z 483 is not accompanied by a satellite signal at m/z 482 representative for H/D back exchanges with the residual water in the linear ion trap meaning that such HDX process does not take place. This means that H/D back exchanges observed for product ions related to the Pgly_{d3} loss (m/z 329 \rightarrow m/z 328 at ActTime = 500 ms and m/z 329 \rightarrow m/z 328 \rightarrow m/z 327 at ActTime = 3000 ms) occur during its storage. In contrast, H/D back exchanges for the ions related to the formal benzyl amine radical loss (consecutive to the prompt CO_2 loss) are not detected. However, very weak m/z 331 signal due to the expected $\text{C}_6\text{H}_5\text{CH}\cdot\text{ND}_2$ radical loss is accompanied by much more intense unexpected signal at m/z 330 related to the $\text{C}_6\text{H}_5\text{CD}\cdot\text{ND}_2$ release. This behavior is reinforced at ActTime = 3000 ms [Scheme 1].

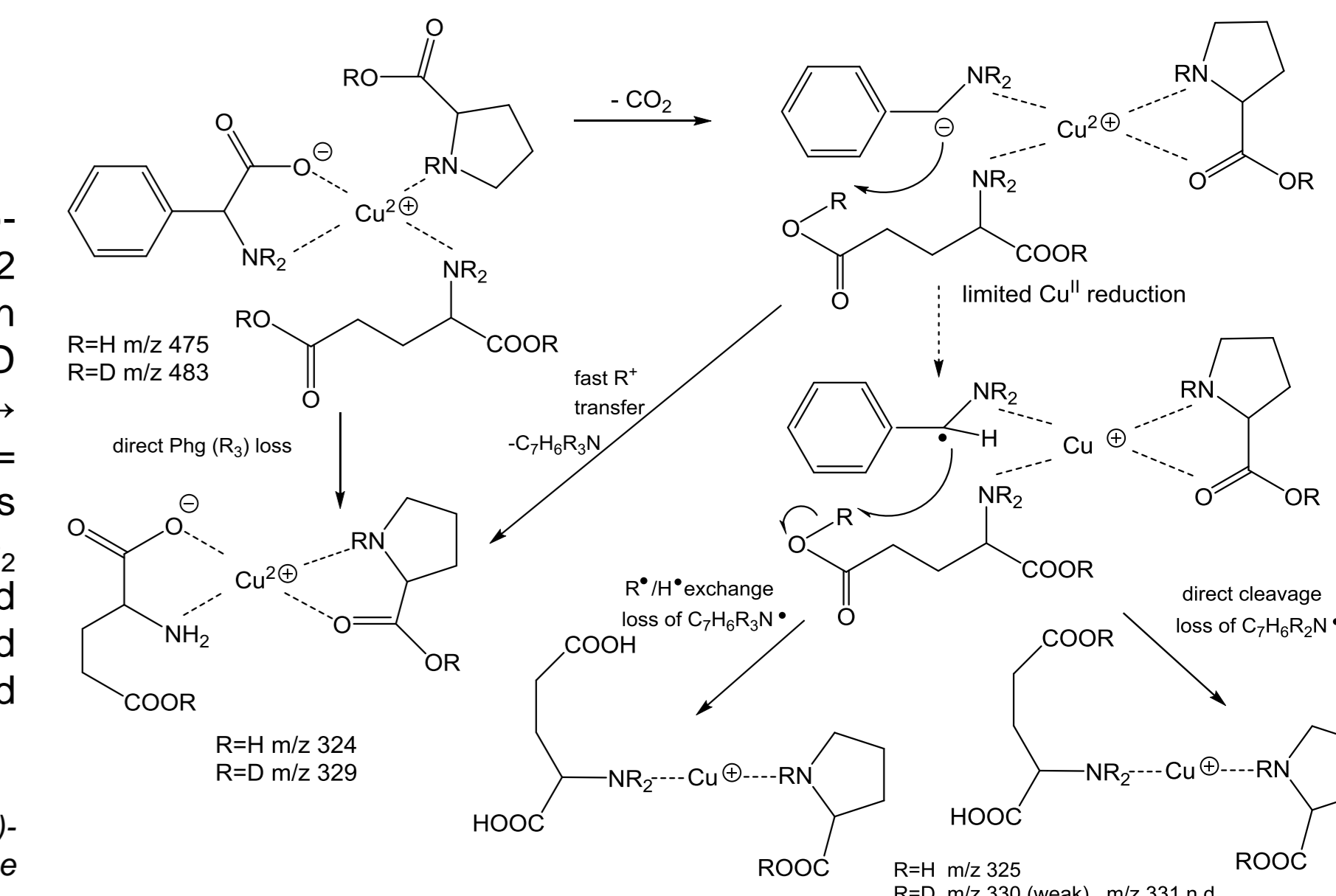
Figure 7. (a) Comparison of region of interest of the product ion spectra of the homochiral $[(^L\text{Pgly}_{d3}+^L\text{Pro}_{d2}+^L\text{Ile}_{d3})-\text{D}+\text{Cu}^{\text{II}}]^+$ CQC ions (m/z 459) activated during 10 ms and 3000 ms in CID mode at NCE = 10% and (b) plots of the $(I_{313}+I_{314})/I_{312}$ and I_{313}/I_{314} ratios versus the ActTime values from 10 to 3000 ms.



The labeled CQC ions with Ile as expected present a larger reduction yield. It increase also with the ActTime values. In addition, the abundance ratio of m/z 314 ion and m/z 313 ion due to the competitive $\text{C}_6\text{H}_5\text{CH}\cdot\text{ND}_2$ and $\text{C}_6\text{H}_5\text{CD}\cdot\text{ND}_2$ losses from Cu^{II} reduction is roughly constant when the ActTime is increased.

CONCLUSION

Under resonant excitation (CID mode), a significant enantioselective Cu^{II} reduction occurs when the side chain of the AA partner in $^L\text{Pgly}/^L\text{Pro}/^L/D\text{AA}/\text{Cu}^{\text{II}}$ complex is nonpolar. The reduction yield and its enantioselectivity are strongly weakened in the case of polar AAs as Glu and Asp. The H^{\cdot} mobility at the side chain of these AAs has been shown to cause hindered reduction. Indeed, the enantioselective reduction of complexes where all mobile protons are deuterated mainly results in the unexpected losses of $(\text{CO}_2+\text{C}_6\text{H}_5\text{CD}\cdot\text{ND}_2)$ rather than $(\text{CO}_2+\text{C}_6\text{H}_5\text{CH}\cdot\text{ND}_2)$ by an unusual stepwise mechanism. In the case of AA=Glu, a particular conformation of the side chain can be reached to promote a reversible D transfer preventing the chiral effect.



Scheme 1. Proposed competitive direct Pgly and stepwise $[\text{Pgly-H}]^{\cdot}$ losses: unexpected $\text{H}^{\cdot}/\text{D}^{\cdot}$ radical exchanges and origin of the enantioselective reduction decrease.

Reference

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