Electronic Supplementary Information for Metallomics

Electronic Supplementary Information

The role of copper in cysteine oxidation: study of intra- and inter-molecular reactions in mass spectrometry

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The reactions between copper ions and cysteine-containing peptides were also analysed in presence of $CuSO_4$ and the results are summarized in Table S1. These measurements were also followed along the time and the conversion rates were in a steady state since the beginning of the experiments.

Table S1 Summary of the cysteine-containing peptides oxidations and complexations in presence $CuSO_4$

		m/z / Th	$CuSO_4$
AIKCTKF	M_1	810.3	+
	Oxidation to cystine ($2M_1$)	540.3/809.4/1617.6	++ / + / +
	Oxidation to $RSOH/RSO_2H/RSO_3H$	826.3/842.3/858.3	– / – – / –
	Copper adducts on M_1	934.1	2 Cu^+ (–)
		871.1/872.2	1 Cu^{2+} (–) / 1 Cu^+ (+)
	Copper adducts on dimer	839.7/840.3	1 Cu^{2+} (–) / 1 Cu^+ (–)
		1678.3/1679.3	1 Cu^{2+} (–) / 1 Cu^+ (–)
ALRCTCS	M_2	753.3	–
	Oxidation to cystine (M_{2ox})	751.1	++
	Oxidation to RS_2O/RS_2O_2	767.3/783.2	+ / ++
	Dimer ($2M_2$)	1503.3	–
	Copper adducts on M_2	939.0	3 Cu^+ (++)
		877.1	2 Cu^+ (+)
		815.2	1 Cu^+ (–)
	Copper adducts on dimer	– –	– –
KCTCCA	M_3	628.3	–
	Oxidation to cystine (M_{3ox})	626.3	++
	Oxidation to $RSOH/RSO_2H/RSO_3H$	644.1/660.1/676.1	– –
		642.1/658.1/674.1	– / + / +
	Dimer ($2M_{3ox}/M_3+M_{3ox}$)	1249.2/1251.2	+ / +
	Copper adducts on M_3	875.9	4 Cu^+ (–)
		813.9	3 Cu^+ (+ +)
		750.0	2 Cu^{2+} (+) (or 2 Cu^+ (+) on M_{3ox})
		688.1	– –
	Copper adducts on M_3+M_{3ox}	1560.7	5 Cu^+ (–)
		1498.7	4 Cu^+ (–)
		1436.9	3 Cu^+ (–)
		1375.0	2 Cu^+ (–)
		1313.1	1 Cu^+ (–)

One-cysteine-containing peptide: AIKCTKF

The peak pattern was slightly different than the one obtained with a Cu electrode (see difference between Table 1 and Table S1) and the main compounds observed in the mass spectrum were the multi-charged dimers. In addition to the oxidation possibly induced by the electrode and oxygen, Cu^{2+} catalyzes the oxidation of cysteine.

Two-cysteine-containing peptide: ALRCTCS

The results obtained in presence of $CuSO_4$ showed only the oxidized peptide (in different forms) and the relative abundance of M_2 was weak. Moreover, Cu^+ ions (up to 3) binds to M_2 (*m/z* = 815.2 Th, 877.1 Th and 939.0 Th) and $[M_2 + 3Cu^I - 2H]^+$ was the most abundant. The tandem mass spectra (data not shown) of these compounds indicate that Cu^+ ions are bound to the lysine and the cys4 for one ion, to arginine and cys4 for two ions, and that the third Cu^+ ions can be either linked to the *N*-terminus or the cys6.

Three-cysteine-containing peptide: KCTCCA

In presence of a copper(II) salt the oxidation of M_3 to M_{3ox} (at the same position than the experiments performed with a Pt or a Cu electrode) gave a conversion rate of ~82% and the oxidation of RSH to RSOH, RSO_2H and RSO_3H was also observed. Of course and as it was observed for ALRCTCS, the addition of oxygen could take place over the sulfur atoms of the cystine (see middle right on Scheme 3). The fragmentation of $2M_{3ox}$ shows the same fragments than those obtained with the copper electrode and neither bridge between two other cysteines than cys2 (the other being oxidized *via* intra-molecular reaction) nor linkage *via* three disulfide bridges are possible.

Finally, Cu^+ was also observed on M_3 with a rather higher abundance than the electrogenerated one and $[M_3 + 3Cu^+ - 2H]^+$ was again the most abundant. However, Cu^+ adducts (up to 5) were also present on the covalent dimer $M_3 + M_{3ox}$ with rather weak relative abundance (see Table S1), which was not observed with the Cu electode.