Electronic Supplementary Information

Strategy for absolute quantification of proteins: CH_3Hg^+ labeling integrated molecular and elemental mass spectrometry

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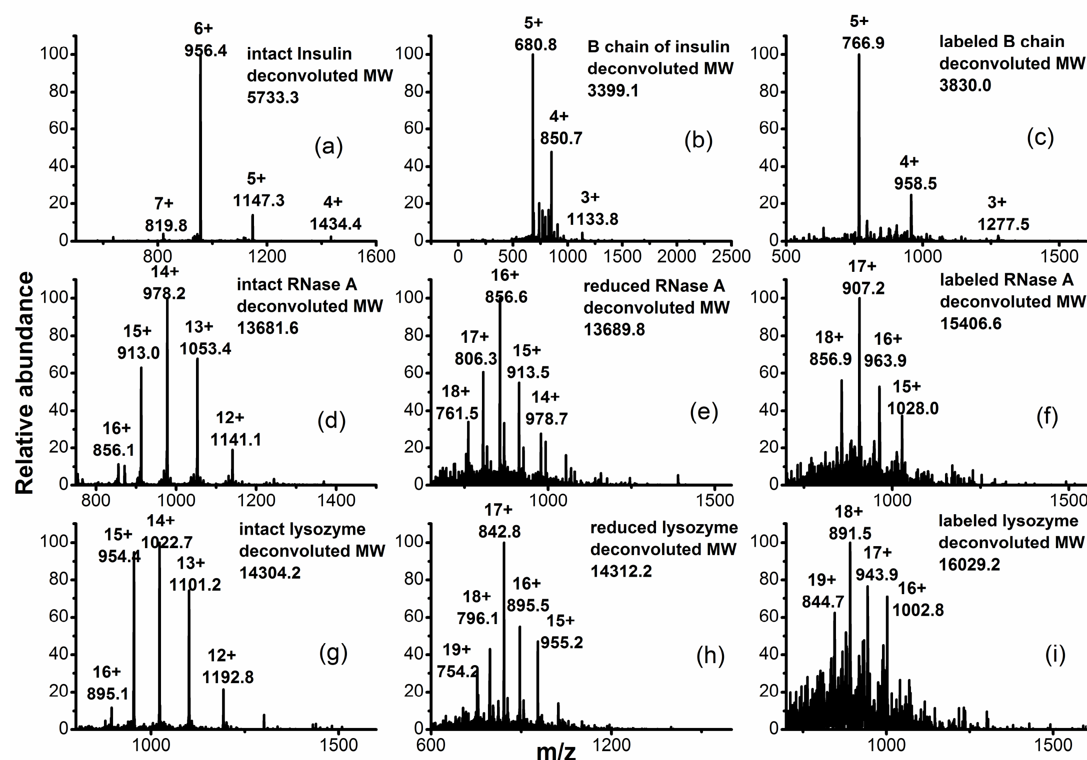

Figure S1 ESI-MS spectra for confirming the binding stoichiometry of the labeled proteins. (a), (d), and (g) are the mass spectra of intact insulin, RNase A, and lysozyme, respectively; (b), (e), and (h) represent those of the reduced proteins by TCEP; (c), (f), and (i) are those of the labeled proteins.

All MS experiments were performed in the positive ion mode. The operational parameters were as follows: nebulizer, 22 psi; dry gas, 12 L/min; dry temperature, 300 °C; capillary, -3500 V; end plate offset, -500 V; skim 1, 35.0 V; skim 2, 6.0 V; capillary exit offset, 60.0 V; octopole, 2.80 V; lens 1, -5.0V; lens 2, -60.0 V; trap drive, 55.0; and max accumulation time, 50 ms.

Although the binding stoichiometry of the labeled protein formed between

CH_3Hg^+ and -SH theoretically reach 1:1 when all –SHs are exposed, it is necessary to confirm it by MMS for an accurate quantification. Our experiment results showed that the realistic performance of CH_3Hg^+ was consistent with theory quite well. Given excessive CH_3Hg^+, all of –SHs (including reduced from –S-S-) could completely react with CH_3Hg^+ due to the high affinity and the specificity of S-Hg reaction. The ESI-MS results of some typical proteins chosen as models including insulin, RNase A, and lysozyme, which contain increasing various –S-S- linkages, are shown in Figure S1. Insulin is a 5.8 KDa dual-chain hormone which contains interchain disulfide bonds (ACys7-BCys7, ACys20-BCys19) and intrachain disulfide bond (ACys6-ACys11).[1] Figure S1a shows the mass spectrum of the intact insulin. Figure S1b is the mass spectrum of B chain of insulin containing two free –SHs after reduced by TCEP. The –SHs of B Chain were then labeled by CH_3Hg^+. The mass shift of 430.9 Da (that is just $2 \times CH_3Hg^+$) could be clearly observed as shown in Figure S1c. RNase A is a 124-residue enzyme that contains four interweaving disulfide bridges (Cys26-Cys84, Cys40-Cys95, Cys58-Cys110, Cys65-Cys72) (Figure S1d).[2] Figure S1e shows the mass shift of 8.2 Da, indicating that the four –S-S- of RNase A were completely reduced to eight –SHs. The mass shift ($8 \times CH_3Hg^+$) (Figure S1f) indicated that all of –SHs exist in the reduced RNase A were completely labeled by CH_3Hg^+. Lysozyme contains 8 cysteines, giving rise to four –S-S- (Cys6-Cys127, Cys30-Cys115, Cys64-Cys80, and Cys76-Cys94).[3] The mass spectrum of intact lysozyme is shown in Figure S1g. Figure S1h and Figure S1i show the mass spectrum of reduced and labeled lysozyme, respectively. The mass shift ($8 \times CH_3Hg^+$) confirmed that the 8 free -SHs in the reduced lysozyme were all labeled. Since the ionizability of labeled proteins decreases with increasing CH_3Hg^+ content, the mass spectra of labeled protein look much noisier than the original ones of the unlabeled protein. However, it can still serve for the determination of the binding stoichiometry.

References

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