Supporting Information

Rapid Purification and High-Resolution Top-down Mass Spectrometric Characterization of Human Salivary α-Amylase

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Figure S1. High-resolution MS analysis of intact purified HSAMY using (A) method 1 based on size-sieving effect, (B) method 2 based on reversed-phase chromatography, and (C) method 3 based on enzyme-substrate specific interaction between amylase and glycogen. Details of methods 1-3 are described in Materials and Methods section.
Figure S2. Determination of reproducibility of glycogen purification method (Method 3) followed by high-resolution MS analysis of HSAMY. (A) Instrument reproducibility, with three injections from the same purification. (B) Purification reproducibility, with three different purifications from the same saliva sample using the glycogen method.
Figure S3. (A) Isolation of a single charge state (55+) of non-reduced HSAMY ions, and (B) ECD and (C) CAD spectra of the isolated ions. The product ions in (B) and (C) were assigned according to the sequence of HSAMY with the removal of the first 15 N-terminal amino acids and the formation of pyroglutamic acid at the new terminus.
Figure S4. Schematic drawings of the possible S-S formation among first five cysteines for non-glycosylated HSAMY assuming that C1 and C5 are involved. (A), (B) S-S between C1-C2 and C4-C5/C3-C5. (C), (D) S-S between C1-C4 and C2-C5/C3-C5. (E), (F) S-S between C1-C3 and C4-C5/C2-C5. (G), (H) S-S between C1-C5 and C2-C3/C2-C4. The amino acid residues for the five cysteines involved are: C1(Cys28), C2(Cys70), C3(Cys86), C4(Cys103), C5(Cys115).
Figure S5. CAD/ECD characterization of partially reduced HSAMY with two S-S reduction. (A) High-accuracy mass measurement of the charge state 55+ precursor ions from partially reduced HSAMY indicating 4 Da increase from the non-reduced HSAMY. (B) Representative CAD/ECD fragment ions from partially reduced HSAMY. (C) Fragmentation map of CAD/ECD spectra of partially reduced HSAMY, suggesting that the second (C2-C5) and fourth S-S (C8-C9) were reduced.