Methods

I. 1D SDS PAGE

4% Stacking gel

10% Separate gel

In situ alkylationed with acrylamide.

Gel stained with silver staining

II. in gel digestion (Trypsin)

Cut a band and cut small pieces around 1mm square

Washed with water(500ul) at 37 degree -10min, 5 times

Destained with 100 ul of **15mM Potassium ferricyanide, 50mM Sodium thiosulfate** at 37degree-10 min

Dehydrated with CH3CN (100ul) at 37 degree-10min

Evaporated with a concentrator

Digested with trypsin (**100mM NH4HCO3 pH8.5, 0.001%(w/v) Trypsin**)

The gels were covered with the trypsin solution at 0degree -45min.

37 degree - overnight

Extracted (1) with **50%CH3CN,0.1%TFA** at 37 degree -10 min

Extracted (2) with **15%isopropanol, 20%formic acid, 25%CH3CN** at 37 degree -15min

Extracted (3) with **80%CH3CN** at 37 degree -10min

Sup (1), (2), (3) and (4) were evaporated.

III. LC-ESI-MS-MS

LC: DiNa(KYA TECH)

Column: Magic C18 0.1mm ϕ x 50mm

Flow: 0.3 $\mu \ell$／min

Solvent A: 0.1%HCOOH, Solvent B: 0.1%HCOOH/90%CH3CN

Gradient: 0%B(30min) → 1%/min → 80%B

MS: QSTAR (ABI)

Scan range: m/z 310-2000

Spray V: 1800V

Curtain gas: N2, 10

Collision gas: N2, 5

Collision energy: 20-50ev

Determination of C terminal peptide

			A	B
	m/z	MW		
N-terminal	601.3	1200.6	GSH(M+16)AQNITAR	GSH(M+16)AQNITAR
Modification	599.86	1197.7	IGEPLVL(K+28)CK	IGEPLVL(K+28)CK
	503.81	1005.57	GAP(K+28)KPPQR	GAP(K+28)KPPQR
	572.82	1143.62	LEW(K+28)LNTGR	LEW(K+28)LNTGR
Insert	901.96	1801.92	-	VYQIAPGKPEIVDSASE
1.C-terminal	866.45	1730.90	-	VYQIPGKPEIVDSASE
	946.43	945.43	-	PEIVDSASE
2.C-terminal	882.98	1763.97	-	VWEPVPLEEVQLVVE
3.C-terminal	650.83	1299.65	AVSISIIEPGEEG	AVSISIIEPGEEG

We digested the protein with trypsin, but we found that C terminal amino acids were not K or R peptides.

Therefore, we determined C terminal peptide of the protein.