Supplementary material

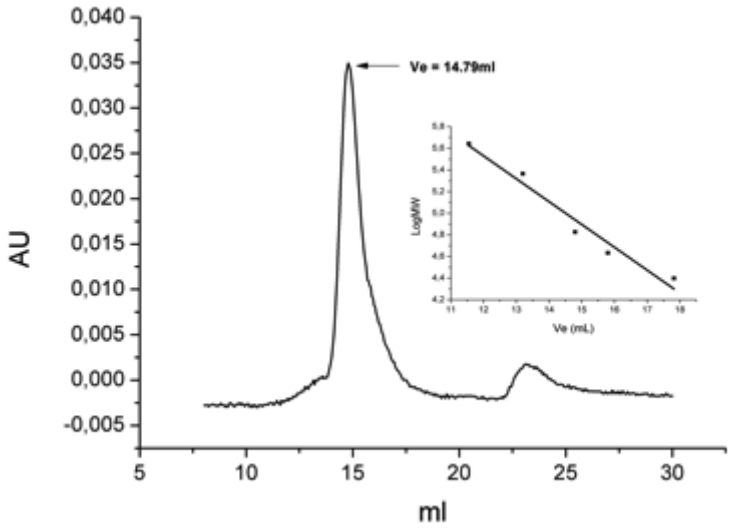
CysK2 is an O-Phospho-L-Serine Dependent S-Sulfocysteine Synthase in *Mycobacterium tuberculosis*

Eva Maria Steiner[a], Dominic Böth[a], Philip Lössl[a], Francisco Vilaplana[b], Robert Schnell[a] and Gunter Schneider#[a]

a. Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

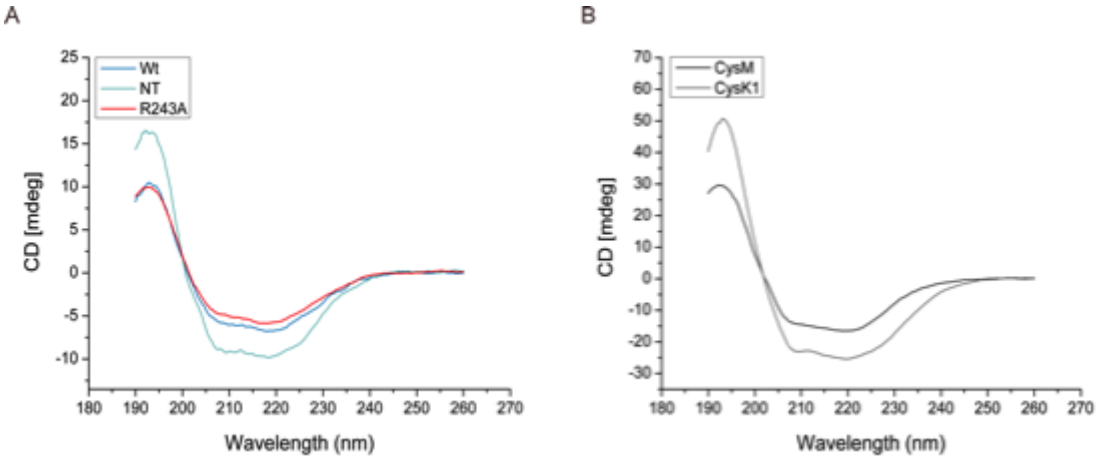
b. Division of Glycoscience, School of Biotechnology, Royal Institute of Technology, Stockholm, Sweden

Supplementary Figure S1:



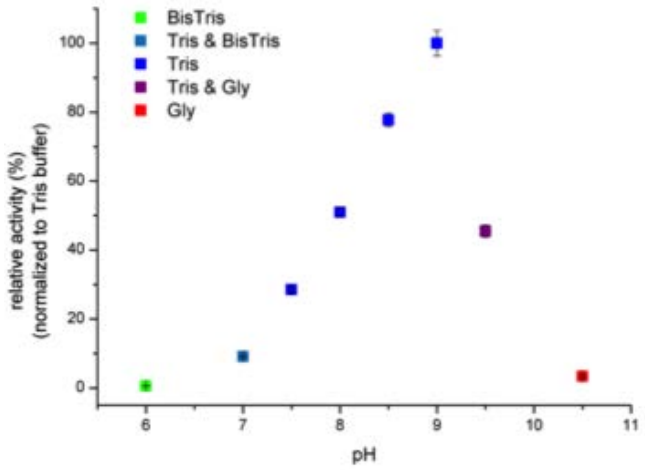
Supplementary Figure S1. The oligomerization state of CysK2 in solution. Analytical size exclusion chromatography was used to estimate the molecular weight of CysK2 in solution. The superdex-200 10/300 column (GE-Healthcare) was equillibrated with the buffer 25 mM Tris-HCl pH 8.0, 150 mM NaCl and 200 µg CysK2 was loaded and eluted at 14.8 ml volume. Using the calibration curve based on chymotrypsinogen-A (25 kDa), ovalbumin (43 kDa), albumin (67 kDa), catalase (232 kDa) and ferritin (440 kDa) the estimated molecular weight is 86.8 kDa. Considering the sequence-derived mass of a monomer (40.1 kDa), CysK2 is most likely a dimer in solution.

Supplementary Figure S2:



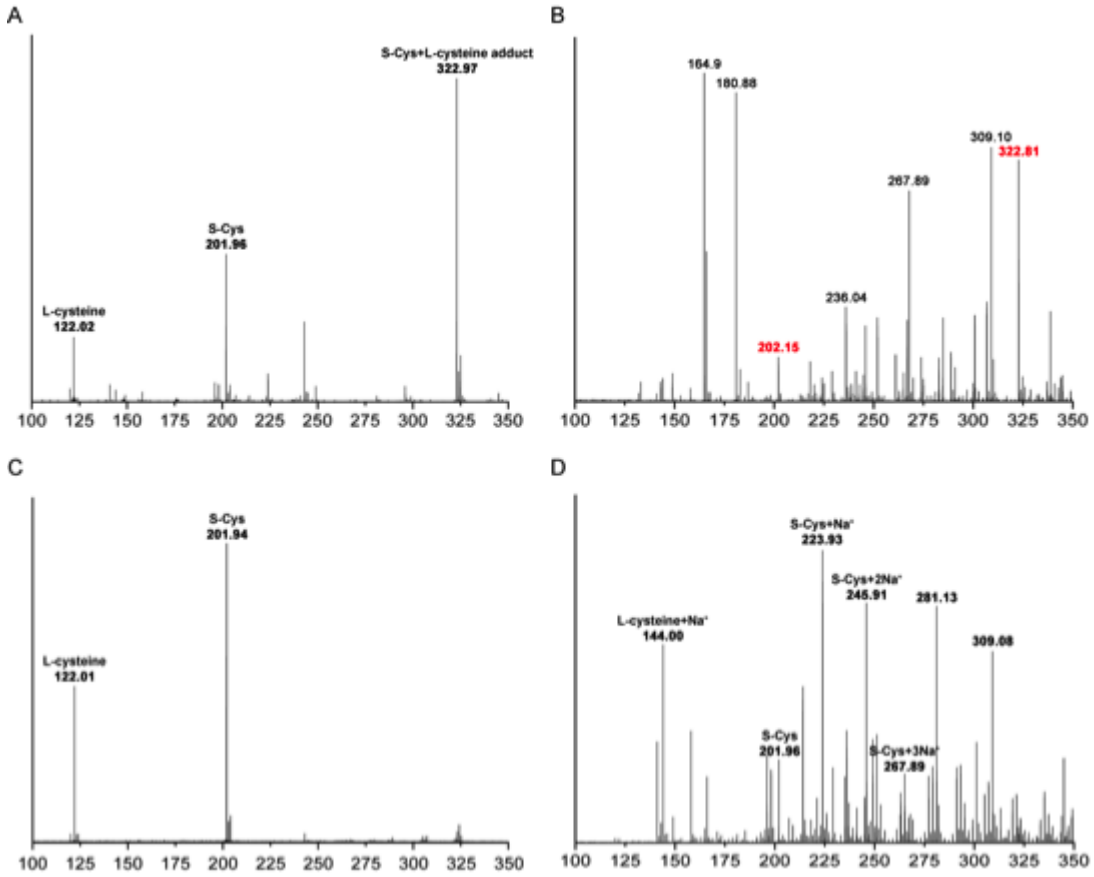
Supplementary Figure S2. Folding integrity of CysK2 investigated by circular dichroism (CD). (A) CD spectra of CysK2, $CysK2_{NT}$ (residues Thr20-Ser353) and the $CysK2_{R243A}$ mutant. (B) For comparison the CD spectra of $CysK1_{Mtb}$ and $CysM_{Mtb}$ are shown.

Supplementary Figure S3:



Supplementary Figure S3. The effect of pH on the rate of phosphate release in first half reaction of CysK2. The pH dependence of the CysK2 catalyzed reaction with OPS using the phosphate-release based assay in the absence of the second substrate reflects the pH-sensitivity of the aminoacrylate intermediate. The buffers used are indicated.

Supplementary Figure S4:



Supplementary Figure S4. ESI-MS and ESI-MS/MS spectra of S-sulfocysteine and the reaction product of the CysK2 reaction with OPS and thiosulfate as substrate. (A) ESI-MS spectra of a commercial sample of S-sulfocysteine with corresponding m/z peaks of 122.02, 201.96 and 322.97, which refer to L-cysteine, S-sulfocysteine (S-Cys) and the S-sulfocysteine-L-cysteine adduct, respectively. (B) Mass spectrum of the reaction product formed by CysK2 using OPS and thiosulfate as substrates. A m/z of 202.15 for S-sulfocysteine and a m/z of 322.81 for the S-Cys+L-cysteine adduct was identified (red). (C) Confirmation of the S-Cys+L-cysteine adduct by fragmentation of the 322 m/z peak, which resulted in peaks for L-cysteine (122.01) and S-sulfocysteine (201.94). (D) Mass-spectrum of S-sulfocysteine in 10 mM Na_2HPO_4 buffer pH 7.0, resulting in ionised molecule fractions due to the presence of sodium cations.

Table S1. Primers for PCR amplification of the CysK2 variants carrying the R243A mutation and the N-terminal truncation.

Oligo name	Sequence (5' -> 3')	Comment
ESOLI_001	CGCGTCGAACAGGCTGATG**GCC**GGGCTGGGCTCGAGTATTTAT	R243A mutagenesis, forward
ESOLI_002	ATAAATACTCGAGCCCAGCCC**GGC**CATCAGCCTGTTCGACGCG	R243A mutagenesis, reverse
ESOLI_005	CATG**CATATG**ACTCCTGGCCGGAATCGCC	Amplification primer for CysK2-NT, forward, NdeI site
SROLI-193	TGCTAGTTATTGCTCAGCGGTGGC	Amplification primer for CysK2-NT, reverse, pET28a specific