Enzymatic Deglutathionylation to Generate Interleukin-4 Cysteine Muteins with Free Thiol

Viswanadham Duppatla*[†], Maja Gjorgjevikj[†], Werner Schmitz[†], Mathias Kottmair[‡], Thomas D. Mueller[‡], and Walter Sebald[†]

Affiliations:

[†] Lehrstuhl für Physiologische Chemie II, Theodor-Boveri-Institut für Biowissenschaften (Biozentrum) der Universität Würzburg, Würzburg, Germany.

[‡] Lehrstuhl für Botanik I-Molekulare Pflanzenphysiologie und Biophysik, Julius-von-Sachs-Institut für Biowissenschaften (Biozentrum) der Universität Würzburg, Würzburg, Germany.

Running title: Enzymatic deglutathionylation of IL-4

Contents:

Table S-1. DNA Sense (S) and Antisense (AS) Primers Used for Mutagenesis of IL-4 cDNA

IL4-S16C	S	5′-CAAAACTTTGAACTGCCTCACAGAGC-3’
IL4-S16C	AS	5′-GCTCTGTGAGGCAGTTCAAAGTTTTG-3’
IL4-N38C	S	5′-GCTGCCTCCAAGTGTACAACTGAGAAGG-3’
IL4-N38C	AS	5′-CCTTCTCAGTTGTACACTTGGAGGCAGC-3’,
IL4-H74C	S	5′-CACAGCAGTTCTGCAGGCACAAGC-3’
IL4-H74C	AS	5′-GTGCCTGCAGAACTGCTGTGCAG-3’,
IL4-Q78C	S	5′-CAGGCACAAGTGTCTGATCCGATTC-3’
IL4-Q78C	AS	5′-CGGATCAGACACTTGTGCCTGTG-3’,
IL4-R81C	S	5′-CAAGCAGCTGATCTGTTTCCTG-3’
IL4-R81C	AS	5′-GTTTCAGGAAACAGATCAGCTGC-3’

Table S-2. Mass Spectrometry Analysis of Purified Glutathione-Modified IL-4 Cysteine Muteins. Deconvoluted spectra as shown in figure 2 were evaluated.

	glutathione-modifed			glutathione-modified	unmodified
IL-4 protein	theoretical	observed	difference		
	molecular mass [Da]			relative amount [%]	
IL4WT	n.d.	n.d.	--	0	100
S16C	15409.754	15409.907	0.153	100	0
N38C	15382.765	15382.871	0.106	97	2.7
H74C	15359.749	15359.974	0.225	97	2.6
Q78C	15368.749	15370.420	1.671	100	0
R81C	15340.706	15340.799	0.093	98	2.1

Table S-3. Mass Spectrometry Analysis of Purified NEM-Labelled IL-4 Cysteine Analogues. Di-NEM conjugates were not observed (see figure S-5).

	Unmodified			with one NEM		
IL4 mutein	theoretical	observed	difference	theoretical	observed	difference
	Molecular mass [Da]					
S16C	15104.708	n.d.		15229.755	15230.744	0.989
N38C	15077.697	n.d.		15202.744	15202.626	-0.118
H74C	15054.681	n.d.		15179.728	15179.712	-0.016
Q78C	15063.681	n.d.		15188.729	15189.802	1.073
R81C	15035.639	n.d.		15160.686	15161.706	1.020

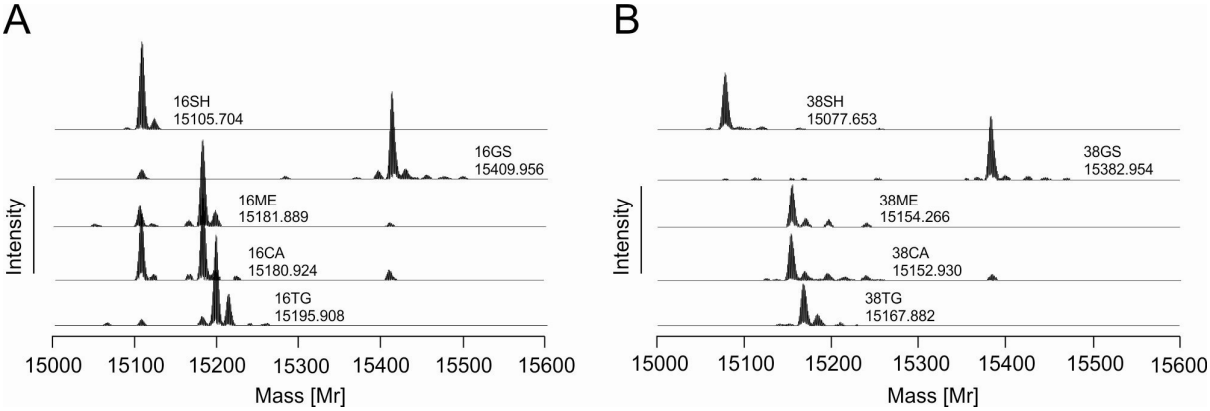

Figure S-1. Mass spectrometry analysis of IL-4S16C mutein (A) or IL4N38C mutein (B) refolded in the presence of 5 mM glutathione (GSH), β-mercaptoethanol (ME), cysteamine (CA), or thioglycolate (TG). The proteins were purified as described under EXPERIMENTAL PROCEDURES. As a reference IL4S16C SH (16SH) and IL4N38C SH (38SH) reduced enzymatically (see figure 7) are included (uppermost trace, each A and B).

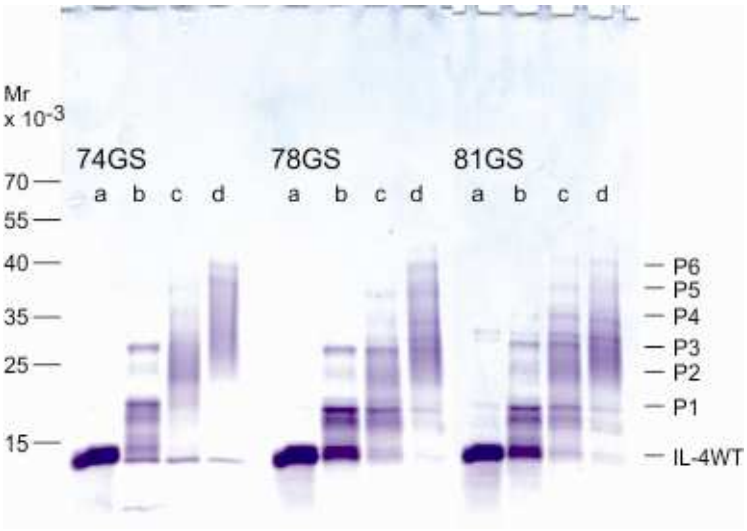

Figure S-2. Chemical reduction of 50 µM IL-4 proteins 74GS, 78GS, and 81GS with DTT at concentrations of 50 µM (lanes b), 100 µM (lanes c), and 150 µM (lanes d). The controls without DTT reduction are shown in lanes a. After chemical reduction proteins were maleimide-PEGylated and analysed by SDS PAGE (see METHODS) using 4 – 20% polyacrylamide gradient gels. The same aliquot of the reaction mixture containing 3.8 µg IL-4 protein was loaded on each lane.

Figure S-3. Time course of reduction of IL-4 wild type and IL-4 GS analogues 16GS, 38GS, 74GS, 78GS, and 81GS with 150 µM DTT for 0, 2, 6, and 12 hours. After chemical reduction proteins were maleimide-PEGylated and analysed by SDS PAGE (see METHODS) using 4 – 20% polyacrylamide gradient gels. The same aliquot of the reaction mixture containing 3.8 µg IL-4 protein was loaded on each lane.

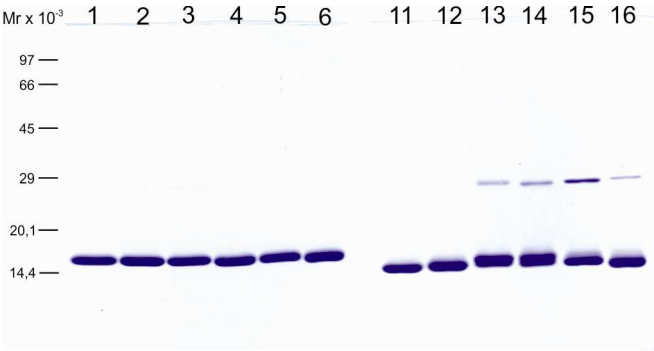

Figure S-4. SDS PAGE analysis of IL-4 cysteine muteins with a free thiol group under reducing (lanes 2 – 6) and non-reducing (lanes 12 – 16) conditions. Lanes 1,11 IL-4WT; lanes 2,12 16SH; lanes 3,13 38SH; lanes 4,14 74SH; lanes 5,15 78SH; and lanes 6,16 81SH.

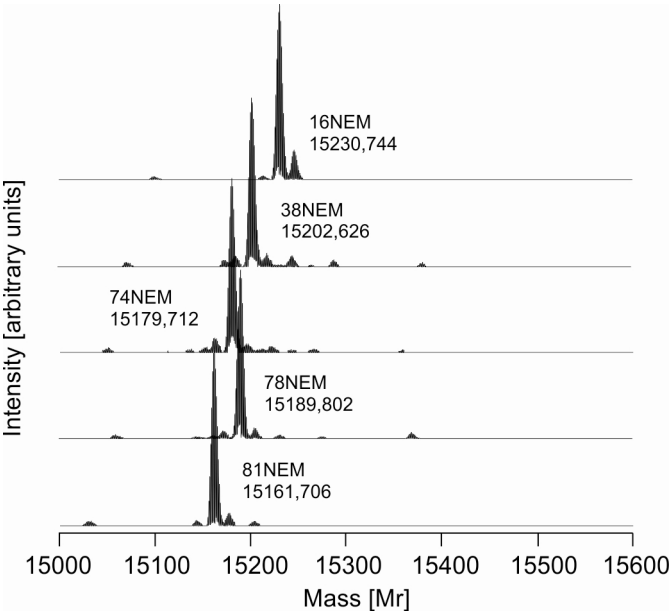

Figure S-5. Mass spectrometry analysis of NEM labelled IL-4 cysteine muteins.