

Oxidative Folding Studies of a Modified Form of Macrophage Colony Stimulating Factor β (M-CSF β)

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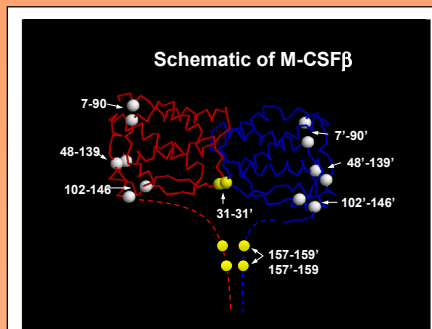
Overview

Novel Aspect: The description of a novel mechanism of subunit dimerization in the refolding pathway of human macrophage colony stimulating factor β (M-CSF β)

M-CSF β : Cytokine responsible for proliferation of monocyte/macrophage cells; 49kD homodimer that contains 9 disulfide bonds; model protein for oxidative folding studies

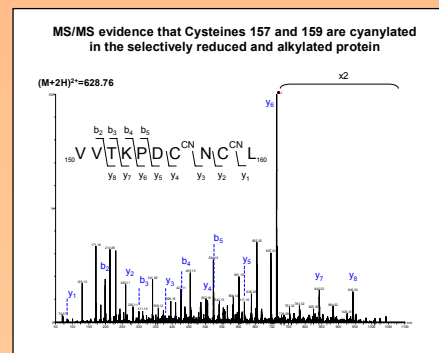
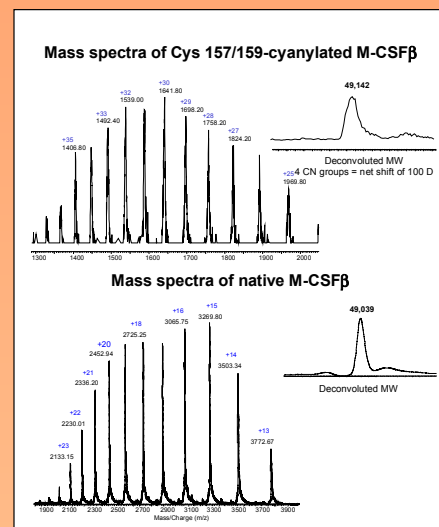
Methods: Selectively cyanylate cysteines known to be involved in dimerization (Cys 157/159) to determine whether alternative mechanisms are possible; ESI-MS/MS and MALDI-ToF/MS/MS used to analyze refolded protein structures

Conclusions: Dimer formation in the refolding pathway of M-CSF β is possible in the absence of Cys 157/159 disulfide bonds; an intersubunit disulfide bond involving Cys 31/31' forms early in the alternate refolding pathway described here

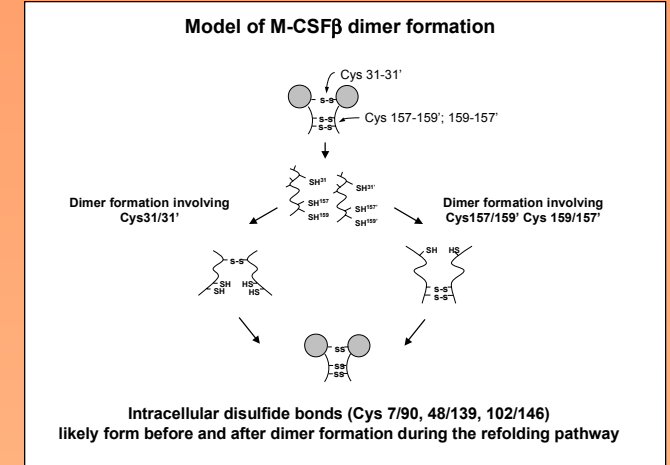
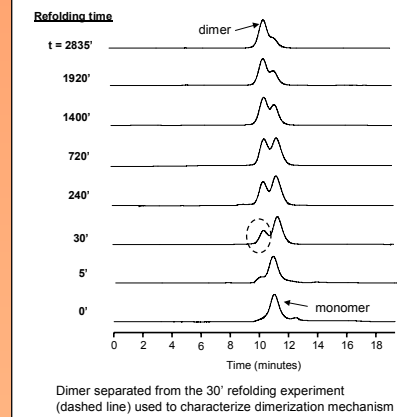


Adapted from the crystal structure of M-CSF α : Pandit et.al. (1993), dashed lines represent residues 149-221 that are present in the β form

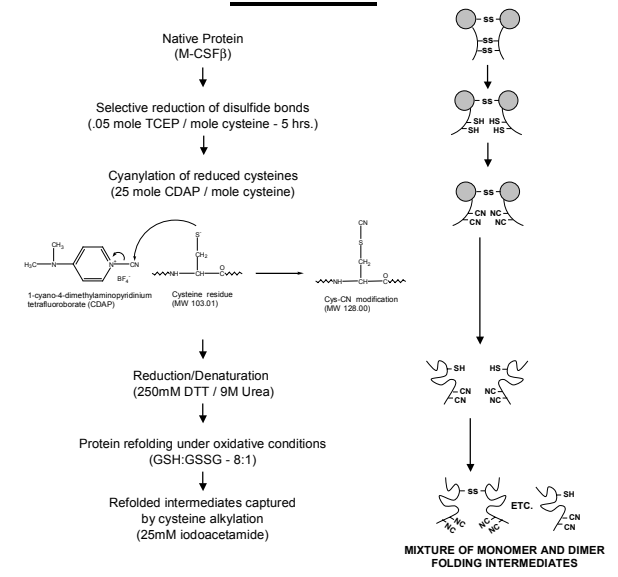
Results



Size Exclusion Chromatography of Cys157,159-CN M-CSF β indicates dimer formation during refolding



Methods



M-CSF β sequence showing peptic peptides

⁴SEYCSH M ¹¹IGSGHLQSLQ ²¹RLIDSQMETS
³¹CQITFEFVDQ ⁴¹EQLKDPVCYL ⁵¹KKAFLLVQDI
⁶¹MEDTMRFRDN ⁷¹TPNAIAIVQL ⁸¹QELSLRLKSC
⁹¹FTKDYEEHDK ¹⁰¹ACVRFYETP ¹¹¹LQLEKVKNV
¹²¹FNETHNLLDK ¹³¹DWNIFSKNCN ¹⁴¹NSFAECSSQD
¹⁵¹VVTKPDCNCL ¹⁶¹YPKAIPSSDP ¹⁷¹ASVSPHQPLA
¹⁸¹PSMAPVAGLT ¹⁹¹WEDSEGETGS ²⁰¹SLLPGEQPLH
²¹¹TVDPGSAKQR ²²¹P

Instrumental Analysis

Protein mass spectra obtained on PE-Sciex API III triple quadrupole or a Micromass Q-ToF Ultima, both equipped with an electrospray inlet.
 Tandem mass spectra (MS/MS) obtained on the Q-ToF or an Applied Biosystems 4700 Proteomics Analyzer (MALDI ToF/ToF)

Conclusions

- In the absence of Cysteine 157/159 disulfide bonds, the protein is able to dimerize and refold toward the native structure
- M-CSF β dimer formation can be mediated through the formation of disulfide bonds between either cysteines 157/159 or cysteine 31/31'

Acknowledgments

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References

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