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Impact of S-sulfocysteine on fragments and trisulfide bond linkages in monoclonal antibodies

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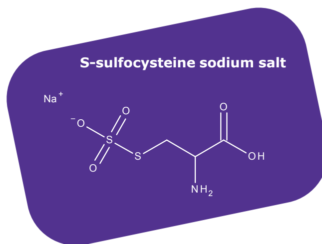
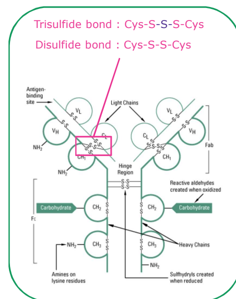
Introduction

The quality of recombinant proteins such as monoclonal antibodies produced using Chinese hamster ovary (CHO) cell-based mammalian systems is dependent on many factors, including cell line, process and cell culture media. Due to these factors, the generated product is heterogeneous and may present chemically induced modifications or post-translational modifications that affect antibody stability, functionality and, in some cases, patient safety.

This study demonstrates that S-sulfocysteine, a cysteine derivative, can increase the cell specific productivity in cell lines cultivated with different processes while minimizing trisulfide linkages in generated mAbs, mainly between heavy and light chain. The supplementation of a cell culture feed with S-sulfocysteine also proved to be useful to reduce the percentage of antibody fragments generated from the monoclonal antibody. Overall, this new component used in the upstream process allows a reduction of product heterogeneity.





Materials and Methods

Cell culture conditions :

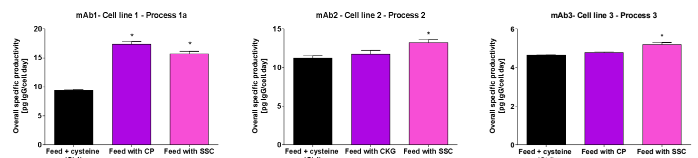
mAb	Cell line	Isotype	Process	FB platform	Scale	Culture conditions	Seeding (c/mL)	Feeding Process
mAb1	CHO-K1	IgG1	Process 1a	Cellvento™ CHO-220	1.2 L bioreactors	37 °C (day 0 - day 5), 33°C (day 5-21), pH 6.95 ± 0.15, 50 % dissolved oxygen, agitation 140 rpm	2.10^5	Main feed: 3% (day 3), 6% (days 5, 7, 9, 14) Glucose adjusted on demand to 6 g/L
mAb1	CHO-K1	IgG1	Process 1b	Cellvento™ CHO-220	1.2 L bioreactors	37 °C, pH 7.0 ± 0.02, 50 % dissolved oxygen, agitation 140 rpm	2.10^5	Main feed: 3% (day 3, 5), 6% (day 7), 3% (day 10, 12)) Glucose adjusted on demand to 6 g/L
mAb2	CHO-K1	IgG1	Process 2	Cellvento™ CHO-220	50 mL spin tubes	37 °C, 5% CO_2, 320 rpm, 80% humidity	2.10^5	Main feed: 3% (day 3), 6% (days 5, 7, 10, 14) Glucose adjusted on demand to 6 g/L
mAb3	CHO-DG44	IgG1	Process 3	Cellvento™ CHO-210	50 mL spin tubes	37 °C, 5% CO_2, 320 rpm, 80% humidity	3.10^5	Main feed: 6% (day 3, 5, 7, 10, 12) Glucose adjusted on demand to 6 g/L
mAb4	CHOZN®	IgG1	Process 4	Excell® Advanced	50 mL spin tubes	37 °C, 5% CO_2, 200 rpm, 80% humidity	3.10^5	Main feed: 5% (day 3), 10% (day 6), 5% (day 8, 10) Glucose adjusted on demand to 6 g/L

Trisulfide bond characterization: Trisulfide bonds were characterized using nano LC-MS after alkylation (NEM) and Lys-C / trypsin cleavage. The separation was performed on a HSS T3 column (Waters) and the analysis was performed using a Q-Exactive Plus mass spectrometer.

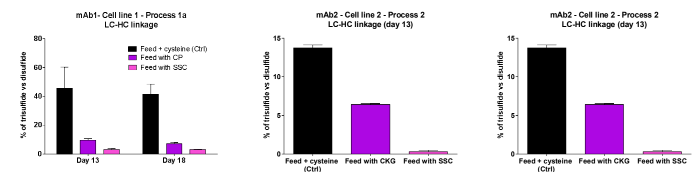
IgG fragmentation : Fragments were separated according to their size in non-reduced conditions using capillary gel electrophoresis with SDS (CE-SDS) and the Beckman Coulter IgG Purity / Heterogeneity assay. 100 µg of IgG were alkylated using 12.5 mM iodoacetamide, denatured 10 min at 70°C 500 rpm and separated using 50 µm fused silica capillary on a CESI 8000 instrument.

Results

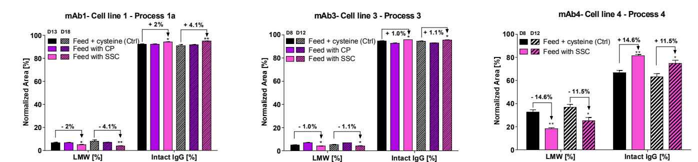
Four suspension CHO cell lines engineered to produce human monoclonal IgG1 antibodies were cultivated using different fed-batch processes. In the control condition, cysteine was added throughout the process using a cysteine-containing feed. In the second condition, cysteine was allowed to interact with pyruvate or α-ketoglutarate in the feed, allowing formation of the thiazolidines CP or CKG respectively[1]. These condensation products can stabilize cell culture media formulations containing cysteine[2] and have been described as a tool to reduce trisulfide bond formation in IgG[3]. They were used in this study as positive control. In the third condition, the cysteine present in the feed was replaced by SSC. This molecule has been described previously as a potent cysteine derivative having an anti-oxidative effect in CHO-based fed-batch processes[4].



Whereas the cell specific productivity (Qp) was only significantly increased for CP with cell line 1, the Qp was significantly increased in the SSC containing process for three cell lines producing different mAbs (Mann-Whitney test). This indicates that SSC can robustly increase Qp in different CHO cell lines or processes.



The starting relative percentage of trisulfide bonds was different for each antibody ranging from 11% to 46%. The use of SSC-containing processes allowed a drastic reduction of the trisulfide bond levels to values below 5%. The effect of SSC was always stronger when compared to the thiazolidine control. Finally, the use of a SSC-containing feed decreased the occurrence of low molecular weight (LMW) fragments with a concomitant increase in the main intact IgG, confirming the positive correlation between trisulfide bonds and fragmentation proposed by Liu et al[5].



Conclusion

Altogether, our results indicate that SSC can be used in cell culture processes to reduce mAb heterogeneity, in particular to decrease the amount of trisulfide bonds and fragments. This cysteine derivative may also be used to develop neutral pH feeds and to increase cell specific productivity while reducing the overall IgG heterogeneity[6].

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