Microbial Cell Factories

Poster Presentation

Cell culture efforts to reduce glycation in recombinant humanized antibody

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Background

Glycation is a common post-translational modification of proteins, resulting from the chemical reaction between reducing sugars such as glucose and the primary amino groups on protein [1]. This non-enzymatic glycosylation reaction generates structural heterogeneity in recombinant IgG_1 antibodies produced by cell culture processes [2]. Recent analytical characterization of a full-length humanized antibody secreted by Chinese Hamster Ovary (CHO) cells revealed that glycation of this protein occurs predominantly at lysine 49 on the light chain of the antibody [3]. This finding contrasts with historical data that have suggested that glycation sites are typically located randomly at all accessible lysine residues distributed over the entire molecule [2,3].

The glycated species accounted for 40–50% of the total antibody produced by transient CHO cell transfections in bioreactors. By contrast, other recombinant antibody molecules produced by CHO cultures generally showed only ~5% glycation [3]. This work documents cell culture process development efforts taken to reduce glycation of this antibody in stably expressing CHO cell lines.

Results

Early stable antibody expression by different CHO clones in 60 mm plates demonstrated no significant differences in glycation (see Table 1). This supports the expectation that glycation of this antibody is an extracellular event such that glycation levels should depend on cell culture conditions and should not vary from clone to clone.

Antibody glycation was ~40–60% in 40L bioreactors and ~10–15% in 1L spinners at the time of harvests (see Table 2). Since the extent of this chemical modification should increase with reaction time and substrate availability, the considerable disparity in glycation between bioreactor and spinner samples is attributed partly to the differences in cultivation time and glucose concentrations.

The results from plate and spinner experiments show that the glycation of this antibody can be reduced to below 40%. Hence, 2L bioreactor experiments were conducted to test the feasibility of lowering glycation by reducing the glucose concentration in the culture medium. The pH, dissolved oxygen, and temperature profiles were controlled identically in all the 2L bioreactors. The extent of glycation in the bioreactor samples collected at the end of culture (day 14) was determined using a boronate affinity chromatographic method previously described [3].

In the first series of bioreactor experiments, the glucose concentration in the batch feed was lowered by ~67%, and the amount of supplemental glucose added was reduced by ~50%. These modifications lowered glycation to 14–20% for each of the three antibody stably-expressing clones tested (see Table 3). Subsequent bioreactor

Table 1: Glycation of antibody produced by different CHO clones in 60 mm plates.

Clone	2	25	92	131	169	191	260	274	277
Glycation (%)	19	21	20	18	19	17	18	18	20

Table 2: Glycation of antibody produced in 40L bioreactors and 1L spinners.

Vessel	Volume	Clone	Harvest Time	Final [Glucose]	Glycation
Bioreactor	2 x 40L	2	Day 11 and Day 14	> 9 g/L	42%
Bioreactor	40L	2	Day 14	> 10 g/L	58%
Bioreactor	40L	2	Day 14	> 7 g/L	42%
Spinner	1L	2	Day 5	< 6 g/L	13%
Spinner	1L	191	Day 5	< 6 g/L	11%

Table 3: Glycation of antibody produced in the first, second, and third series of 2L bioreactor experiments. Each condition was evaluated in duplicate bioreactors. Data represents average ± standard deviation obtained from duplicate cultures.

Experiment	Clone	Condition	Glycation	Final [Glucose]
1	2	Control	19 ± 2 %	2.9 ± 1.1 g/L
1	191	Control	17 ± 2 %	1.9 ± 0.4 g/L
1	274	Control	15 ± 1 %	1.4 ± 0.5 g/L
2	2	Control	16 ± 2%	1.8 ± 0.1 g/L
2	2	Partial Continuous Glucose Feed	10 ± 1%	0.8 ± 0.1 g/L
3	2	Control	19 ± 1%	2.2 ± 0.2 g/L
3	2	Full Continuous Glucose Feed	6 ± 0 %	0.5 ± 0.4 g/L

experiments employed clone 2 exclusively, and this reduced glucose feed process was used as the control.

In the second set of bioreactor experiments, antibody glycation was further reduced to ~10% (see Table 3) by eliminating glucose from the batch feed and replacing it with a continuous glucose feed. In the final round of bioreactor experiments, by using glucose-free inoculation medium and batch feed, and by implementing a continuous glucose feed strategy to maintain glucose at even lower concentrations throughout the culture, the glycated species was minimized to 6% (see Table 3). Despite the variation in antibody glycation, product titers and cell-specific productivities were comparable in all the 2L bioreactor experiments.

Conclusion

The percentage of glycated antibody was reduced by lowering the glucose concentration in the culture medium. The extent to which glucose concentration in the cultures was controlled directly impacted the antibody glycation level.

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