COMBINED EFFECT OF AMMONIA STRESS AND CELL LINE AGE ON CHO CELL DERIVED VRC01 MONOCLONAL ANTIBODY GLYCOSYLATION

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Glycosylation is a critical post-translational modification of proteins in mammalian cells. Glycosylation of monoclonal antibodies (mAbs) is of particular interest due to the effect it has on the effector function, structure and stability of the mAb, thus making it an important product quality attribute. To achieve a desired therapeutic effect, it is necessary to produce an antibody with a specific glycosylation profile. To produce this antibody, a better understanding of how changes to culturing conditions impact the glycoforms of mAbs is required. Ammonia concentrations typically increase during mammalian cell cultures, mainly due to glutamine and other amino acid consumption. In this work, we detect significant changes to glycosylation following ammonia stress of CHO-produced VRC01, a broadly neutralizing antibody undergoing clinical trials to treat HIV. Ambr250 bioreactors were run in parallel with varying concentrations of ammonia stress on CHO cells of two cell line ages. To determine the glycan profiles, the culture supernatant was collected over the course of the run and VRC01 was purified using gravity flow chromatography with protein A resin. Glycans were fluorescently labeled and released from the mAb and eluted from a BEH Amide column using a Waters AQUITY H-class UPLC. We found that increased ammonia concentrations led to a decrease in total and terminal galactosylation, sialylation, and fucosylation of the associated glycans. While age did not have an effect on antibody galactosylation and sialylation under no ammonia stress, reduced galactosylation and sialylation was more severe for the more aged cell line when ammonia stress was present. This observed reduction in sialylation of glycoproteins following ammonia stress is consistent with previously published results [1-2]. Antibody fucosylation was found to be reduced in the more aged cell line in all cases, but ammonia stress did not exacerbate this effect.

1. Nam H.J., et al. The Effects of Culture Conditions on the Glycosylation of Secreted Human Placental Alkaline Phosphatase Produced in Chinese Hamster Ovary Cells. Biotechnol Bioeng.2008, 100, 1178-1192. 2. Chen, P.F., Harcum, S.W., 2005. Effects of amino acid additions on ammonium stressed CHO cells. J. Biotechnol. 2005, 117, 277–286.