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REDUCTION OF METABOLIC WASTE PRODUCTS, AMMONIA AND LACTATE, THROUGH THE COUPLING OF GS SELECTION AND LDH-A DOWN-REGULATION IN CHO CELLS

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The cultivation of Chinese hamster ovary (CHO) cells for the production of therapeutic proteins inevitably accompanies the production of metabolic wastes, mostly ammonia and lactate. Ammonia alters cell growth, productivity and the glycosylation patterns of proteins, and lactate acidifies culture media, having negative effects on cell culture.

A stable CHO cell line should be established for the manufacturing process of therapeutic proteins, and the development of stable cell lines is usually based on two expression systems: the dihydrofolate reductase (DHFR) system and the glutamine synthetase (GS) system. Compared to the DHFR system, the GS system produces a reduced level of ammonia because the GS protein uses ammonia to produce glutamine. In order to overcome the lactate accumulation problem, down-regulation of the lactate-producing enzyme, lactate dehydrogenase-A (LDH-A), has been shown to be effective. Engineering of the LDH-A gene has been applied for several CHO cell lines with the DHFR system, but there has been no trial which couples the ammonia reduction from the GS system and lactate reduction through cell engineering.

In the present study, the GS system was used for the expression of therapeutic antibody in CHO cells, thereby reducing ammonia in the culture media. In addition, the LDH-A gene was down-regulated with shRNA to reduce lactate production. The antibody-producing cell line produces a reduced level of ammonia compared to the host cell line due to the over-expression of the GS protein. The down–regulation of the LDH-A gene in the antibody-producing cell line not only reduces the level of lactate but also further reduces the level of ammonia, accomplishing complete waste reduction. LDH-A down-regulation was also applied to the host cell lines of the GS system – the CHO-K1 cell line and the GS deficient CHO-K1 cell line. However, LDH-A down-regulated host cells could not survive the pool-selection process. Given that the GS system uses a glutamine-depleted condition as a form of selection pressure, enhanced glycolysis is inevitable and the down-regulation of LDH-A appears to hinder metabolic changes.

Taken together, the application of LDH-A down-regulation in the producing cell line of the GS system successfully reduced both ammonia and lactate levels. However, LDH-A engineering could not be applied to the host cell lines because it inhibits the selection process of the GS system.