Development of Super-K562 Cells Producing Erythropoietin in Glucose-Free Medium

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ABSTRACT

Lactate and ammonia are two major toxic waste products formed during mammalian cell culture. Accumulation of the side products have negative effects of on cell growth and specific production rate. In this study, K-562 cells were used as the host cell of a recombinant protein. Effects of carbon sources were invetigated focused on the cell culture span, the accumulation of lactate and ammonia in culture of recombinant K-562 cells.

INTRODUCTION

Mammalian cells are increasingly used in the production of proteins for therapeutic uses because many human proteins of the investigative and clinical value require folding events and post-translational modifications, such as glycosylation, which are unable to be occurred in microorganisms. Accumulation of noxious by-products and exhaustion of nutrients are important factors in mammalian cell cultures. The major waste products are ammonia and lactate. Lactate is produced mainly from glycolysis for energy acquisition and ammonia is from glutamine metabolism, which is excreted when amino acids are transported into the cell. Lactate causes cessation of cell growth if accumulation of lactate goes beyonds the buffering capacity of a medium. Accumulation of ammonia resulted in the inhibition of cell growth, decrease in production rate, and specific alterations of protein glycosylation[3]. Because of these factors, the cell density in conventional batch culture is low and the culture span is short. Consequently, product concentration is low, which is undesirable for production process. In this work, our objective is to decrease the formation of lactate and ammonia by using low metabolite producing host cells.

MATERIALS AND METHODS

K-562 (ATCC CCL-243) cells were transfected with EPO gene. K-562 cells

were maintained with Dulbecco's Modified Eagle Medium (DMEM base)(Sigma, USA) supplemented with 5.5 mM glucose, 2 mM glutamine, 3.7 g/L sodium bicarbonate, and 10% fetal bovine serum (FBS)(Gibco, USA). Cell viability was determined by counting in a hemocytometer using the tryphan blue exclusion method. Concentration of lactate, glucose, and glutamine in culture media were measured with YSI model 2700 Biochemical Analyzer (Yellow Springs Instrument, USA). Ammonia was measured with an ammonia assay kit (Wako Pure Chem., Japan).

RESULTS AND DISCUSSION

Cell growth of r-K562 cells producing recombinant human EPO was investigated in batch cultures with glucose, sodium lactate (SL), sodium pyruvate (SP), and methyl pyruvate (MP). Fig.1. shows construction of vectors for EPO gene transfection.

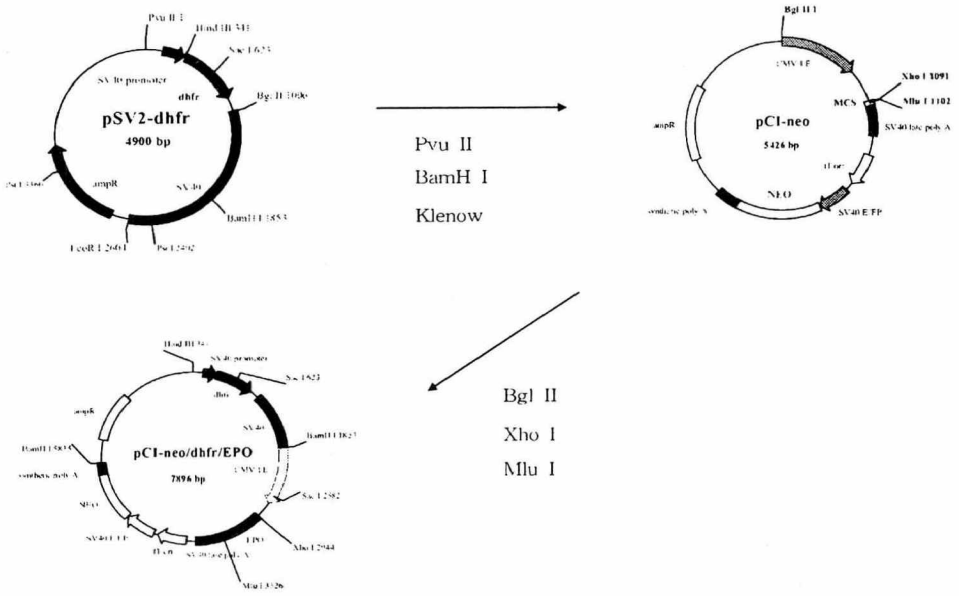

Fig.1. Construction of vector for EPO producing cell.
After dhfr gene was digested by Pvu II, BamH I from pSV2-dhfr, it was made blunt end by klenow. EPO and dhfr genes were ligased to pCI-neo with Bgl II, Xho I, and Mlu I.

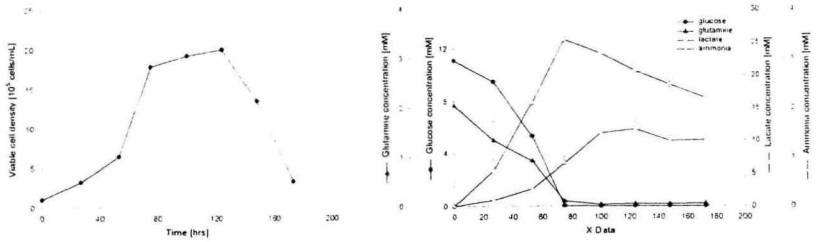

Fig.2. Growth kinetics of CHO-K1 cells in glucose medium.

Cells were cultured in Roswell Park Memorial Institute(RPMI) 1640 Media supplimented with 10% fetal bovine serum (FBS).

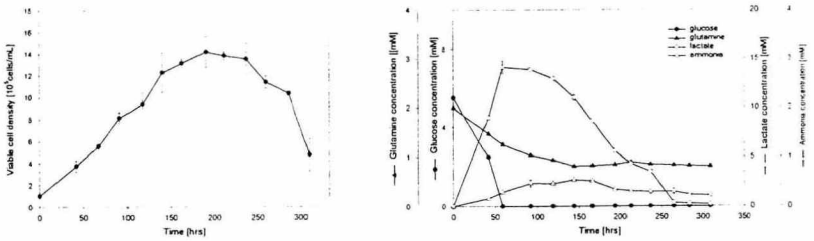

Fig.3. Growth kinetics of K-562 cells in glucose medium.

Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplimented with 5.5 mM glucose and 10% fetal bovine serum (FBS).

Fig.2 and 3 shows the growth of CHO-K1 and K-562 cells in glucose medium.

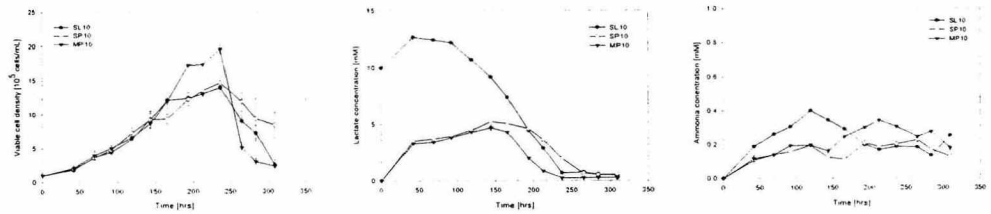

Fig.4. K-562 cell growth in non-glucose media.

Cells were cultured in sodium pyruvate (SP), sodium lactate (SL), and methyl pyruvate (MP) media which are DMEM suplemented with 10 mM of carbon sources and 10% FBS.

Compared with CHO cells, K-562 showed longer culture span. K-562 cells could growth until 200 hrs, which is 2-3 times longer culture span than that of CHO cells. The longer life span of K-562 cells was thought to be caused by the

uptake of lactate after glucose was totally consumed. This result indicates that K-562 cells may utilize lactate as energy sources. The possibility of utilizing other carbon sources of K-452 cells was thus investigated. As shown in figures 4 and 5, K-562 cells could grow with sodium pyruvate, methyl pyruvate as well as lactate. The level of ammonia secretion was decreased with increasing the concentration of carbon sources (data not shown). K-562 is a erythro-leukemia originated suspension cell. This result was supports that K-562 can utilize lactate, pyruvate as energy sources. All together, our results suggest that K-562 cells have appropriate lactate dehydrogenase (LDH), monocarboxylate transporters (MCT), and gluconeogenesis systems to grow on various carbon sources. The suspension characterization of K-562 is another advantage in large-scale animal cell culture. Protein productivity of rK-562 in order to confirm the potential of K-562 cells as a promising host for recombinant protein production in animal cell cultures.

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