Effects of peptone on hybridoma growth and monoclonal antibody formation

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Abstract

Hybridoma WuT3 secreting a monoclonal antibody against T lymphocytes was grown in RPMI 1640 medium supplemented with 1% human serum. The effect of the concentration of peptone, as an additive, was investigated on cell growth, monoclonal antibody formation, and cell metabolism over 0–10 g l\(^{-1}\) range. It was found that 1–5 g l\(^{-1}\) peptone can significantly promote the growth of cells and increase the formation of monoclonal antibody, especially at 3–5 g l\(^{-1}\), when both the accumulating level and secretion rate of monoclonal antibody are higher than that at other peptone concentrations. Based on glucose, lactate and ammonia analysis data, the efficiency of glycolysis was assessed and the utilization of amino acids was more efficient at 3–5 g l\(^{-1}\) peptone. The cell growth and monoclonal antibody formation were inhibited at higher peptone concentrations, e.g. 10 g l\(^{-1}\).

Introduction

Monoclonal antibodies produced by hybridoma cell lines have been widely used for in vivo diagnosis and therapy, in vitro diagnosis, immunoassay, etc. (Zhang and Rong, 1987). The large-scale culture of hybridoma cells is of particular interest in the production of these monoclonal antibodies. To facilitate the purification, serum-free or protein-free media are usually used in hybridoma culture so that the amount of contaminant proteins is reduced. In these media, some additives such as insulin, transferrin, albumin, lipid and trace elements have been used to optimize culture medium compositions (Bols et al., 1988; Shacter, 1987). When hybridomas are grown in these media, cell densities and antibody concentrations are usually lower compared with normal serum concentration (Low and Harbour, 1985). It has also been reported that medium enriched with amino acids is favorable to cell growth (Mather and Tsao, 1992).

Hybridoma WuT3 is a cell line which relies strongly on serum. It cannot grow well in several serum-free media, but has been successfully grown with good monoclonal antibody secretion at low concentrations of human serum (1%). In this study, the effect of peptone concentration has further been investigated on WuT3 cell growth and monoclonal antibody formation.

Materials and methods

Hybridoma cell line

Hybridoma cell line WuT3 used in the study was established by Wuhan Institute of Biological Products, Ministry of Public Health, PRC. It was achieved by a fusion of spleen cells of immunized Balb/c mouse with SP2/0 mouse myeloma cells. Hybridoma cell line WuT3 produces the monoclonal antibody (IgG\(_{2a}\)) against human T lymphocytes, which, in organ transplantation, can be used against rejections.

Medium

The cells were cultured on RPMI 1640 medium (Sigma) with 2 g l\(^{-1}\) D-glucose and 0.3 g l\(^{-1}\) L-glutamine without antibiotics but supplemented with 2 g l\(^{-1}\) sodium bicarbonate, 1% human serum and various levels of peptone.
Human serum

Human serum, AB-type, without virus contamination, was kindly presented by Wuhan Institute of Biological Products, Ministry of Public Health.

Peptone

Peptone (Tryptose, Oxoid) was dissolved in RPMI 1640 medium at 20 g 1⁻¹ and sterilized with 0.22 μm filter.

Cell culture

WuT3 hybridoma cells were grown in medium with 1% human serum and passaged. The cells were inoculated in eight 100 ml-flasks with 10 ml medium and cultured in CO₂ incubator. After cells grew up to middle log-phase, they were collected in a flask as seeding cells. Appropriate amount of ingredients, shown in Table 1, were dispensed into six 175 ml-flasks to form a series of peptone concentrations of 0, 1, 2, 3, 5, 10 g 1⁻¹. The flasks were placed in a 5% CO₂ incubator, at 37 °C, and 4 ml sterile samples were collected in laminar flow hood for cell counting and analysis every day.

Analysis methods

Viable cell density was determined by 0.1% trypan blue vital staining and counting with a hemocytometer. Glucose and lactate were enzymically analyzed (Liu et al., 1992). Monoclonal antibody contents and the amount of ammonia were determined by immunological single diffusion and urea-nitrogen analytic kit respectively.

Results and discussion

In the process of cell growth and metabolism, some amino acids are always rapidly utilized, e.g. glutamine, isoleucine, leucine, lysine, threonine, valine, etc. In the meantime, some other amino acids are frequently produced in the process, depending on the cell lines, such as alanine, glycine, glutamic acid and aspartic acid (Thomas, 1990; Graf and Schugerl, 1991). If these fast-consumed amino acids are supplemented, a marked increase in cell density and monoclonal antibody production will be achieved (Duval et al., 1991). It was also reported that the overall enhancement of amino acids might lead to heavy cell growth in serum-free culture (Mather and Tsao, 1992).

Peptone is a mixture which contains an amount of amino acids, oligopeptides, vitamins and trace elements, and has been used in animal cell culture (Keay, 1975, 1976, 1977). In our experiment, it was found that addition of peptone resulted in above 20% increase of cell densities, but the change of peptone concentrations over the range of 1-5 g 1⁻¹ had little effect on cell densities. More concentrated peptone, for instance, 10 g 1⁻¹, was unfavorable to cell growth (Fig. 1). This was probably due to the inhibition to cell growth by some components in peptone.

Although the effect of amino acid concentrations so far has not been fully confirmed on the synthesis and accumulation of monoclonal antibody proteins, the monoclonal antibody formations in culture supernatant would generally increase with the enhancement of cell densities regardless of the proportion between monoclonal antibody concentrations and cell densities. In this study, monoclonal antibody formation was always improved at 1-5 g 1⁻¹ peptone, especially at 3 or 5 g 1⁻¹ peptone (Fig. 2). Only at 3 or 5 g 1⁻¹ peptone, the highest antibody level occurred on sixth day during 7-days culture while the highest antibody levels were attained on the last day at other peptone concentrations. Moreover, the peptone concentrations had little effect on specific monoclonal antibody formations. But it is also found that after the addition of lower concentrations of peptone (1-2 g 1⁻¹), the specific antibody formation decreased slightly while the antibody secreting levels were still enhanced. Nevertheless, at higher peptone concentrations (3-10 g 1⁻¹), the specific antibody formation still continued at high levels. At 10 g 1⁻¹ peptone level, although the specific...