Cultural and physiological factors affecting expression of recombinant proteins

J.B. Griffiths and A.J. Racher
CAMR, Porton Down, Salisbury, Wiltshire SP4 OJG, UK

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Abstract

The variability in expression of recombinant proteins has been analyzed with regard to (a) comparison of clones from the same transfection experiment; (b) comparison of the same genetic construct in different cell lines; (c) the effect of the culture system used (free suspension, aggregate suspension, and microcarrier); and (d) physicochemical parameters in long-term (100d) culture in a macroporous fixed bed bioreactor (FBR).

Differences in product expression between clones were accompanied by differences in growth rates, metabolic kinetics, and ability to grow in suspension as opposed to attached culture. The single most important factor affecting product expression when comparing constructs (for SEAP and IgG), cell lines (BHK 21 and myeloma), and culture systems was whether cells were grown in an attached or suspension mode. Thus key factors could be related to cell morphology (suspension versus monolayer), the presence of microenvironments and physiological stress to control growth rate.

The relationship of key process parameters to volumetric and specific rAb productivity of the FBR was investigated in a partial factorial experiment with a rBHK cell line. The highest productivity levels are associated with a combination of the highest values tested for re-cycle (195 ml min⁻¹) and dilution rates (1 d⁻¹) and glutamine concentration (2.5 mmol l⁻¹), plus the lowest values for bead size (2 mm) and inoculum density (10⁷ ml⁻¹). Together with data from fluidised bed cultures, these results suggest that higher productivity is not primarily the result of greater cell numbers within the system but more the physicochemical definition of the system.

Abbreviations: FIBR – fluidised bed bioreactor; FBR – fixed bed reactor; STR – stirred tank reactor; SEAP – secreted alkaline phosphatase; rAb – recombinant antibody

Introduction

The use of animal cells for the industrial production of recombinant proteins has increased dramatically over the last ten years. This emphasises the necessity of defining and controlling culture conditions to ensure maximum yields of high quality products. Considerable attention has been paid to understanding the biochemical and physiological factors affecting both growth and production kinetics of cultured animal cells. However, this has been largely restricted to mAb production by hybridomas (e.g. Al-Rubeai et al., 1992). To obtain the full benefit from the use of recombinant DNA technology in animal cells, similar studies need to be made for recombinant cell lines. Reports now appearing in the literature which include studies on the kinetics of recombinant protein secretion are, generally, for a particular product by a given cell line (Cockett et al., 1990; Hayter et al., 1991; Robinson and Memmert 1991; Pendse et al., 1992). There are fewer reports describing the expression of either several recombinant proteins in one cell line or the same recombinant protein in several cell lines, or the use of different culture systems (Wagner et al., 1988; Conradt et al., 1989; Ryll et al., 1990). However these studies do not usually describe the kinetics of product forma-
tion, which has been addressed in this work, and has led to some clear indications of the factors most important for maximising recombinant product expression in cell culture.

Materials and methods

Cell lines and media

The rAb expressed by the cell lines BHK.IgG and F3b10 (derived from BHK21 and Sp2/0 cell lines respectively) consists of the variable portions of a murine antibody, immunoglobulin class IgG1, linked to the constant parts of the human IgG1 molecule (Kaluza et al., 1991). Expression is driven by the murine IgG promoter. The cell line BHK.SEAP expresses the secreted form of alkaline phosphatase. Construction of these cell lines is detailed elsewhere (Racher et al., 1994).

The microcarrier and aggregate culture of the BHK cell lines were done in high glucose DMEM: the FBR and F1BR cultures were with 1:1 DMEM/I-Iams F12. Serum was supplied at 5% v/v. The F3b10 cells were cultured in RPMI 1640 containing the serum-replacement Nutridoma-SR (Boehringer-Mannheim) at 1% v/v.

Culture conditions

Two different STR systems were used in this study. Firstly, spinner flasks (250 ml working volume) (Corning) fitted with a paddle impeller (diameter 5.4 cm; height 1.8 cm) operated at 50 rpm. The spinner flasks were kept in a 7% CO2–93% air atmosphere. The second STR was a 2 l (1 l working volume) vessel (Applikon BV, Schiedam, The Netherlands) fitted with a 4.0 cm diameter, 3.3 cm high impeller. The pH was controlled at 7.1 ± 0.1 and dissolved oxygen at 60% air saturation. The impeller speed was 100 rpm for the F3b10 clone and 60 rpm for BHK.IgG.

The BHK cell lines were grown on Cytodex 3 microcarriers (Pharmacia AB, Uppsala Sweden) at 3 g l⁻¹. The BHK lines were also grown in suspension culture as natural aggregates (Moreira et al., 1992). The F3b10 cell line was only grown as a suspension culture. For both suspension and aggregate cultures, the final viable cell density after inoculation was 1.5–2.0 × 10⁵ cells ml⁻¹.

Design and operation of the FBR with porous Siran (Schott Glaswerke) beads has been described in detail previously (Racher et al., 1990; Racher and Griffiths, 1993). Briefly the FBR was comprised of a 150 ml vessel containing 100 ml of beads. For the FIBR, the reactor vessel volume was 200 ml and contained 80 ml, settled volume, of beads. Both reactor vessels were connected to a 1.5 l (1.0 l working volume) reactor which was used for medium conditioning. The FBR and FIBR were both operated as batch cultures for the first 72–96h before continuous medium feed was started by means of a peristaltic pump. A constant volume was maintained in the reservoir by a weir in the vessel wall. The system was operated in continuous perfusion mode for 240h, or for 2400h.

The characteristics of the borosilicate beads used in the FBR were: diameter, 1–2 or 5–6 mm; porosity, 60%; pore size, < 200 μm. The beads used in the FIBR were smaller, with a diameter of 0.7 mm.

Analytical methods

Cell numbers were determined using a haemocytometer. Viable cells were distinguished by the trypan blue dye exclusion method. IgG1 was measured by ELISA as described previously (Racher et al., 1990), except anti-human Ig F(ab')₂ (Amersham International plc, Amersham, UK) was used with human IgG1 as the standard. SEAP activity was determined spectrophotometrically (Berger et al., 1988).

Results and discussion

1. Clonal variation of Sp2/0 cells expressing SEAP

The variability in both expression of recombinant proteins and a range of cultural characteristics has been analyzed using cell lines from a single transfection experiment. High producing transfectants of Sp2/0 expressing SEAP (including HP62D, HPGC and HP35B) at different maximum cell specific rates were isolated (Hauser et al., unpublished). Additional differences were: the minimum inoculum size needed to initiate a new culture, the serum-dependence of growth and SEAP production, lactate production kinetics (this difference was only found in shake-flask and not in controlled bioreactor cultures) and growth kinetics. This variability, also seen in hybridomas, indicates the potential of screening for useful scale-up culture characteristics as well as productivity levels. One consistent feature (Fig. 1) was the correlation of high SEAP production with high glutamine catabolism and