IFN-γ Glycosylation Macroheterogeneity and Intracellular Nucleotide and Sugar Nucleotide Contents of CHO Cells Cultivated in Batch Culture

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Abstract: We developed an ion-pair RP-HPLC method to measure variations of intracellular nucleotide and sugar nucleotide pools throughout CHO cell cultures producing IFN-γ as a model glycoprotein. The data suggest that UDP-glucose and nucleotide triphosphate availabilities as well as the cell energetic status could influence the macroheterogeneity of IFN-γ glycosylation.

Key words: CHO cells, glycosylation, energy charge, sugar nucleotides,

1. INTRODUCTION

IFN-γ bears two N-glycosylation sites resulting in three possible glycoforms: 2N, 1N and 0N. IFN-γ glycosylation is not constant over the course of CHO cell batch cultures: the proportion of 2N IFN-γ declines with a concomittant increase in the 0N glycoform. Nucleotide and nucleotide sugars could be responsible for that phenomenon as they are carrier molecules of carbohydrates in protein glycosylation processus and energetic molecules. Therefore, an ion-pair RP-HPLC assay, allowing to measure thirteen nucleotides and nucleotide sugars, was developed and applied to monitor these molecules in CHO cell extracts during batch cultures.

R. Smith (ed.), Cell Technology for Cell Products, 435–438.

2. MATERIALS AND METHODS

CHO cells were grown either in serum free RPMI (BSA: 5g.L^{-1}; insulin: 5mg.L^{-1}; transferrin: 5mg.L^{-1}) or RPMI + 10% FCS or PF-BDM, a rich protein-free medium. Batch cultures were performed in 4-L bioreactors (Incelltech SGI), at pH 7.2, 37°C, 50% air saturation and 50 rpm. Cell viability was measured by trypan blue assay. IFN-γ production was determined by ELISA (R&D Systems) and macroheterogeneity was assessed by Western-Blot (Amersham Biosciences). Intracellular nucleotides and nucleotide sugars were analysed by ion-pair RP-HPLC (C18 column Alltech, 254nm, 40°C) after perchloric acid extraction (Ryll *et al*., 91).

3. IFN-γ PRODUCTION AND GLYCOSYLATION MACROHETEROGENEITY

Maximal cell and IFN-γ titers were maximal in rich media, *i.e*. RPMI + 10% FCS and PF-BDM. Maximal IFN-γ concentration was two-fold higher in PF-BDM than in RPMI with or without FCS. In RPMI with or without FCS, 2N IFN-γ proportion decreased during the culture, contrary to PF-BDM.

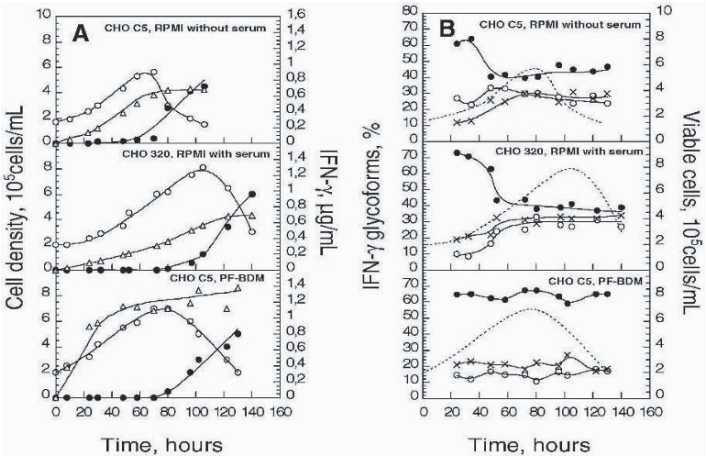

Figure 1. Kinetics of viable cells (A,○), dead cells (A,•), IFN-γ production (A, △), and IFN-γ macroheterogeneity (B, IFN 2N : •, IFN 1N: ○ , IFN 0N: ×, viable cells: dotted line).

4. SUGAR NUCLEOTIDE CONTENTS

UDP-GalNAc and UDP-GlcNAc contents increased with time in the three cultures probably as a consequence of ammonia accumulation (Ryll

et al., 1994). UDP-Glucose titer was maximal simultaneously to the cell specific growth rate, and then decreased. Compared to PF-BDM, UDP-Gal content remained relatively low in RPMI with or without FCS. GDP-Man accumulated throughout the three cultures, meaning that this molecule is not limiting for glycosylation.

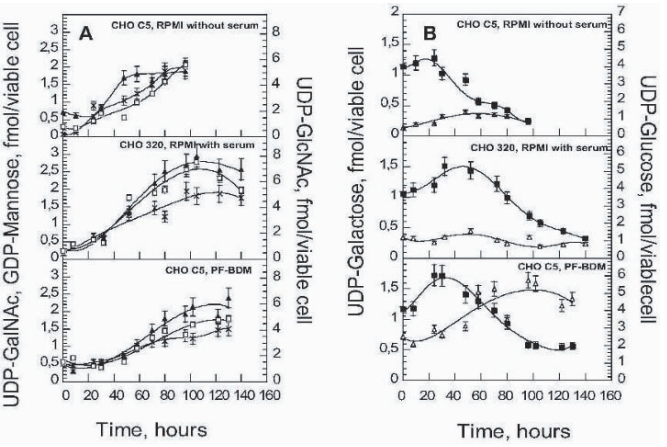

Figure 2. Kinetics of sugar nucleotides during CHO cell batch culture (UDP-Glc: ■, UDP-Gal:△ , UDP-GlcNAc:× , UDP-GalNAc: ▲, GDP-Man: □).

5. NUCLEOTIDE TRIPHOPHATE CONTENTS AND ADENYLATE ENERGY CHARGE

Adenylate energy charge, defined as (ATP+0.5ADP/ATP+ADP+AMP) reflecting the physiological state of the cells, is maintained at high values (around 0.95) as long as IFN-γ is produced in PF-BDM. Therefore, it can be assumed that this medium may supply precursors that are limiting in RPMI with or without FCS, particularly nucleotides and nucleotide sugars, which are crucial for N-glycosylation reactions.

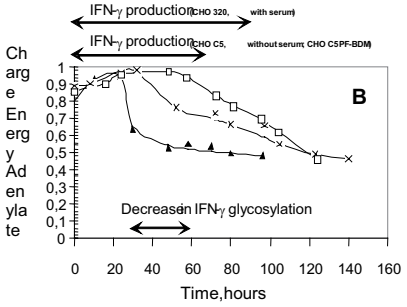

Figure 3. Kinetics of adenylate energy charge during CHO cell batch culture in three different media (RPMI without serum: ▲, RPMI with serum :× , PF-BDM : □).

REFERENCE

Ryll, T., Valley, U., and Wagner, R., 1994, Biochemistry of growth inhibition by ammonium ions in mammalian cells, Biotechnol. Bioeng. **44**:184-193.