Metabolism Kinetics of Glucose in Anchorage-dependent Cell Cultures

SUN Xiang-ming (孙祥明)， ZHANG Yuan-xing (张元兴)

(*State Key Lab. Bioreactor Eng., East China Univ. Sci. & Technol., Shanghai 200237, China*)

Abstract: The kinetic model of glucose metabolism was established and successfully applied to batch cultures of *r*CHO and *r*BHK cells. It was found that a large amount of glucose was utilized for cell maintenance, and the overwhelming majority of maintenance energy from glucose was by its anaerobic metabolism in both *r*BHK and *r*CHO cell cultures. The overall maintenance coefficients from aerobic metabolism were 1.9×10^{-13} mmol/(cell·h) for *r*CHO cells and 7×10^{-13} mmol/(cell·h) for *r*BHK cells. In addition, all $G_{O/T}$ and $E_{O/T}$ gradually increased with the same trend as the cell growth in the culture of both *r*CHO and *r*BHK cells. The overall molecule yield coefficients of lactate to glucose were 1.61 for *r*CHO cells and 1.38 for *r*BHK cells. The yield coefficients of cell to glucose were 4.5×10^{8} cells/mmol for *r*CHO cells and 1.9×10^{8} cells/mmol for *r*BHK cells, respectively.

Key words: animal cell culture; metabolism; kinetics; glucose; maintenance energy

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1 INTRODUCTION

Glucose is one of the major carbon source and energy source in animal cell cultures[1]. The control of glucose at the optimal level is of critical importance for achieving high cell density, product concentration and productivity. This depends largely on the understanding of glucose utilization and regulation in cultured cells.

Glucose is utilized either through the pentose phosphate pathway (HMP) to provide nucleotides and reducing power for biosynthesis, or through glycolysis (EMP) and the subsequent tricarboxylic acid (TCA) cycle to provide metabolic intermediates and energy for cell growth and survival. The metabolic flux distribution of glucose between the two pathways varies with cell strain and culture environment. When glucose is the limiting substrate, more glucose is channeled through HMP[2, 3]. However, when cells grow rapidly at a high glucose concentration, the metabolic flux of glucose through HMP is suppressed and a large amount of glucose is transformed to pyruvate through EMP[3]. In practice, only a small amount of pyruvate is further oxidized to carbon dioxide through the TCA cycle[4]. Under the action of lactate dehydrogenase, a large fraction of pyruvate is transformed to lactate which is the typical end metabolite of aneroblic metabolism in animal cell culture. High yield of lactate to glucose was observed for the batch culture of human FS−4 cells by Glacken et al.[5] and for the continuous culture of hybridomas by Miller et al.[6]. Although alanine can be formed by the transamination reaction between pyruvate and amino acids[7], lactate is the main by-product of the glucose metabolism. Many researchers put emphasis on preventing lactate accumulation in animal cell cultures[5], but little work was done on the contribution of anaerobic

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Biography: SUN Xiang-ming (1969−), male, native of Jiangyan county, Jiangsu province, Dr., assistant researcher, engaged in biochemical engineering.

metabolism in cell growth and maintenance.

Although the glucose metabolism in animal cell culture has also been extensively investigated, most researches were focused on the culture of anchorage-independent cells. The continuous culture mode was adopted in order to obtain kinetic parameters of cell growth and metabolism at the steady state[7–10]. For the anchorage-dependent cells, it is difficult to obtain the metabolism parameters under steady-state conditions because of the unfeasibility of continuous operation. In this work, a kinetic model of glucose metabolism in batch cultures was proposed, and the contribution of metabolic flux of aerobic and anaerobic pathways to maintenance energy is discussed. The results are helpful to understanding the metabolism of anchorage-dependent cells and to optimizing the process control of their large-scale culture.

2 MATERIALS AND METHODS

2.1 Cell Line and Culture Medium

The recombinant CHO cell line (*r*CHO-DEH-I) expressing human EPO and the recombinant BHK cell line (*r*BHK) expressing Factor VIII were kindly provided by Dong-E-E-Jiao Co. Ltd. (Shandong, China) and the Institute of Biochemstry, Chinese Academy of Sciences (Shanghai, China), respectively. The 1:1 mixture of Dulbecco′s modified Eagle′s medium (DMEM, Gibco, USA) and Ham′s nutrient mixture F–12 (Gibco, USA) supplemented with 5% fetal bovine serum (FBS, Gibco, USA) was used for *r*CHO cell culture, and DMEM supplemented with 5% FBS for *r*BHK cells.

2.2 Batch Culture Operation

*r*CHO and *r*BHK cells were subcultured for four generations in a 75 ml T-flask. When growing to confluence, the cells were digested by 0.25 g/L trypsin solution and pipetted to single cell suspension, and then seeded into 14 T-flasks (75 ml) with 8×10^4 cells/ml. All the flasks were inoculated under 5% CO_2 at 37°C. Every 12 h one flask was withdrawn to count the cells and determine the concentrations of glucose, lactate and amino acids.

2.3 Analytical Methods

Viable cells were counted with trypan exclusion using a hemacytometer. Every sample was counted in triplicate to get the average cell density.

Glucose and lactate concentrations were determined using glucose reagent kit (Inst. Biol. Prods. Shanghai, China) and the method of lactic dehydrogenase[6], respectively.

The amino acid concentrations were determined by a reverse phase high pressure liquid chromatograph (HPLC, HP1100, Hewlett Packard, German) with *o*-phthaldialdehyde (OPA) precolumn derivatization[11].

3 RESULTS AND DISCUSSION

3.1 Kinetic Model of Glucose Metabolism

As the main carbon and energy source for cell growth, glucose is utilized for three aspects. One

is for cell proliferation, including the synthesis of cell composition and provision of the energy for cell multiplication. Another is for the production of desired products, e.g., constituting the sugar chain of glycoprotein. The third is for providing the energy for cell maintenance, accompanied by the formation of the by-products such as lactate and alanine. The rate of glucose consumption can be given by:

$$-\frac{\mathrm{d}G}{\mathrm{d}t}=\frac{\mu X}{Y_{\mathrm{G}}}+mX+\frac{\mathrm{d}P}{Y_{\mathrm{p}}\mathrm{d}t}, \tag{1}$$

where

$$\mu=\frac{1}{X}\frac{\mathrm{d}X}{\mathrm{d}t}. \tag{2}$$

The first term of the right side in Eq.(1) represents the consumption rate of glucose utilized for cell growth, the second for cell maintenance and the third for product formation. Generally, the identical cell composition implies the constant requirement of energy and materials. Therefore, the number of glucose molecules needed for forming a new cell remains constant under certain conditions. In other word, the yield coefficient of cell versus glucose (Y_{G}) is a constant.

In EPO molecules, the sugar chain constitutes about 40% of the glycoprotein in weight. Because of very low EPO concentration in the medium, glucose required for EPO synthesis is negligible, so is the energy needed for product formation. Then, the third term of the right side of Eq.(1) can be ignored.

The energy for cell maintenance comes from two routines, i.e., aerobic metabolism and anaerobic metabolism. The proportion of these two parts directly depends on the efficiency of the TCA cycle and the flux of EMP. Generally, the flux of EMP is not regulated in cell culture in vitro[12] and relies on glucose concentration and cellular activity. The pyruvate is always accumulated in the cytoplasm. Because of the excess of pyruvate at the entrance of the TCA cycle, the flux of TCA cycle is controlled by its inherent rate limited by the oxidation of NADH and keeps constant under normal conditions. Therefore, the energy per cell per unit time (m_{O}) obtained from TCA is almost invariant. It was reported that only a small part of pyruvate (about 5%) enters the TCA cycle[4]. A large amount of pyruvate in the cytoplasm pool, which can not enter the TCA, can be transformed to lactate by lactate dehygrogenase, or to methylgloxal by a series of complex reactions[13], or to alanine by transamination[7]. The flux to form methylgloxal is as less as 0.1%~0.5% of the total EMP flux[13], so that it can be ignored in the material balance. On the other hand, the quantity of glucose utilized for cell proliferation is extremely small and is almost channeled through HMP. Only no more than 11% of glucose in this pathway is transformed to pyruvate, which may be further transformed to lactate[2]. The quantity of lactate from this part of glucose is negligible. Therefore, it is reasonable to assume all the lactate coming from glucose metabolism for cell maintenance, not from that for cell growth.

One molecule of glucose can produce two molecules of lactate or alanine, therefore

$$mX=m_{\mathrm{O}}X+\frac{1}{2}\left(\frac{\mathrm{d}P_{\mathrm{Lac}}+\mathrm{d}P_{\mathrm{Ala}}}{\mathrm{d}t}\right). \tag{3}$$

Substituting Eqs.(2) and (3) into Eq.(1) and ignoring the third term of the right side of Eq.(1), we obtain:

$$-\frac{\mathrm{d}G}{\mathrm{d}t}=\frac{1}{Y_{\mathrm{G}}}\frac{\mathrm{d}X}{\mathrm{d}t}+m_{\mathrm{O}}X+\frac{1}{2}\left(\frac{\mathrm{d}P_{\mathrm{Lac}}+\mathrm{d}P_{\mathrm{Ala}}}{\mathrm{d}t}\right). \tag{4}$$

According to Eq.(4), m_{O} can be obtained from the material balance of glucose during the steady phase and Y_{G} from that in the exponential growth phase.

The typical cell growth profiles of *r*CHO and *r*BHK cells in batch cultures are given in Fig.1. Figure 2 shows the glucose consumption and lactate formation corresponding to the cell growth profiles. As it can be seen, during the steady phase the cell density does not vary obviously, and the rates of glucose consumption and lactate formation also slightly decreased as compared with that during the exponential phase. 17.8 mmol/L of lactate is produced by *r*CHO cells with the consumption of 10.7 mmol/L glucose, i.e., the overall molecule yield of lactate to glucose is 1.61. Similar yield coefficients were reported for some hybridoma cells[6]. However, in *r*BHK cell culture, the molecule yield of lactate to glucose is 1.38. These results indicate that the metabolic flux varies remarkably with the cell line despite the fact that glucose may be utilized via the same pathway in these cells.

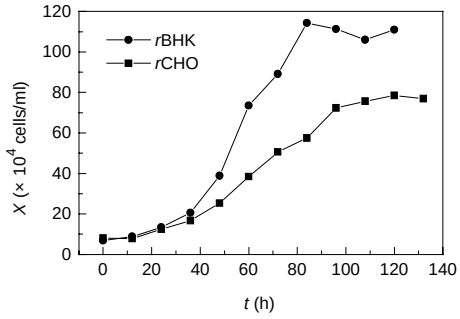

Fig.1 Profiles of cell growth in the batch cultures of *r*CHO and *r*BHK cells

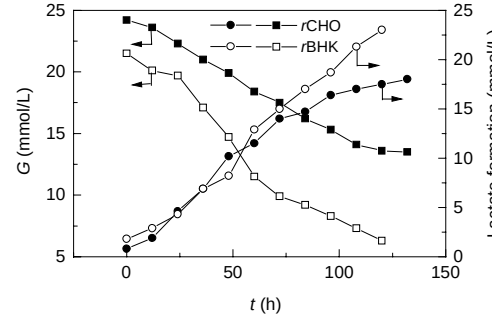

Fig.2 Glucose consumption and lactate formation of *r*CHO and *r*BHK cells in batch cultures

During the steady phase, glucose was mainly used to produce energy for cell maintenance. Because alanine production was no more than 5% of the lactate accumulation, it was reasonable to ignore alanine term in Eq.(4). Ignoring the term of cell growth, it is convenient to obtain m_{O} values of 1.9×10^{-13} mmol/(cell·h) for *r*CHO cells and 7×10^{-13} mmol/(cell·h) for *r*BHK cells, respectively. Joining the cell density, m_{O} and the concentrations of glucose and lactate in Eq.(4), the values of Y_{G}, 4.5×10^{8} cells/mmol for *r*CHO cells and 1.9×10^{8} cells/mmol for *r*BHK cells, are obtained. Hence, different cell lines may have different compositions and need different amounts of glucose for its multiplication and maintenance.

The kinetic equation of glucose metabolism is obtained by replacing Y_{G} and m_{O} in Eq.(4) with calculated values in batch cultures of *r*CHO and *r*BHK cells, respectively. For *r*CHO cells,

$$-\frac{\mathrm{d}G}{\mathrm{d}t}=2.22\times10^{-6}\frac{\mathrm{d}X}{\mathrm{d}t}+0.5\frac{\mathrm{d}(P_{\mathrm{Lac}}+P_{\mathrm{Ala}})}{\mathrm{d}t}+1.9\times10^{-13}X\ , \tag{5}$$

and for *r*BHK cells,

$$-\frac{\mathrm{d}G}{\mathrm{d}t}=5.26\times10^{-6}\frac{\mathrm{d}X}{\mathrm{d}t}+0.5\frac{\mathrm{d}(P_{\mathrm{Lac}}+P_{\mathrm{Ala}})}{\mathrm{d}t}+7\times10^{-13}X\ . \tag{6}$$

The model values of glucose concentrations in batch cultures of *r*CHO and *r*BHK cells could be obtained by taking the experimental data shown in Figs.1 and 2 to Eqs.(5) and (6). It is shown that the kinetic model of glucose metabolism represents satisfactorily the experimental data (Fig.3).

3.2 Structure Analysis of the Energy Metabolism for Cell Maintenance

In consideration of the formation of 36 molecules of ATP by one molecule of glucose through aerobic metabolism, the relationship between the production rate of ATP from aerobic metabolism of glucose and the glucose consumption rate for this part under steady state can be written as

$$\frac{\mathrm{d}A_{\mathrm{O}}}{\mathrm{d}t}=36\frac{\mathrm{d}G_{\mathrm{O}}}{\mathrm{d}t}=36m_{\mathrm{O}}X\ , \tag{7}$$

or

$$(A_{\mathrm{O}})_{n+1}-(A_{\mathrm{O}})_n=18m_{\mathrm{O}}(X_{n+1}+X_n)\Delta t, \tag{8}$$

$$(G_{\mathrm{O}})_{n+1}-(G_{\mathrm{O}})_n=m_{\mathrm{O}}X\Delta t. \tag{9}$$

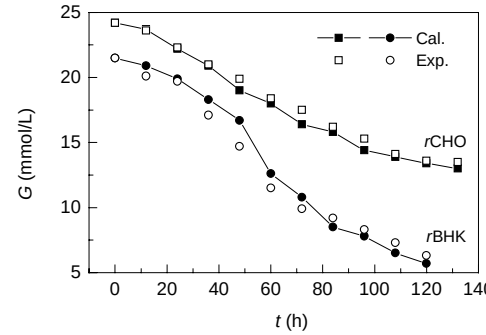

Fig.3 Comparison of glucose concentrations from calculation and experiment in batch cultures of *r*CHO and *r*BHK cells

One molecule of glucose produces two molecules of ATP and two molecules of lactate through anaerobic metabolism in mammalian cells. Similarly, we may deal with the glucose consumption through the anaerobic metabolism and obtain:

$$\frac{\mathrm{d}A_{\mathrm{F}}}{\mathrm{d}t}=2\frac{\mathrm{d}G_{\mathrm{F}}}{\mathrm{d}t}=\frac{\mathrm{d}P_{\mathrm{Lac}}}{\mathrm{d}t}, \tag{10}$$

or

$$(A_{\mathrm{F}})_{n+1}-(A_{\mathrm{F}})_n=(P_{\mathrm{Lac}})_{n+1}-(P_{\mathrm{Lac}})_n\ , \tag{11}$$

$$(G_{\mathrm{F}})_{n+1}-(G_{\mathrm{F}})_n=0.5((P_{\mathrm{Lac}})_{n+1}-(P_{\mathrm{Lac}})_n\ . \tag{12}$$

The ratio of the energy from aerobic metabolism to the total maintenance energy from glucose during t_n~t_{n+1} can be obtained by combining Eqs.(8) and (11),

$$E_{\mathrm{O/T}}=\frac{18m_{\mathrm{O}}(X_n+X_{n+1})\Delta t}{18m_{\mathrm{O}}(X_n+X_{n+1})\Delta t+(P_{\mathrm{Lac}})_{n+1}-(P_{\mathrm{Lac}})_n}. \tag{13}$$

The ratio of glucose consumed through aerobic metabolism to its total consumption for cell maintenance during t_n~t_{n+1} could also be obtained from Eqs.(9) and (12):

$$G_{\mathrm{O/T}}=\frac{m_{\mathrm{O}}(X_n+X_{n+1})\Delta t}{m_{\mathrm{O}}(X_n+X_{n+1})\Delta t+(P_{\mathrm{Lac}})_{n+1}-(P_{\mathrm{Lac}})_n}. \tag{14}$$

Figures 4 and 5 are the profiles of $E_{\mathrm{O/T}}$ and $G_{\mathrm{O/T}}$ during the cultures of *r*CHO and *r*BHK cells, respectively. Obviously, the maintenance energy from aerobic metabolism is much less than that

from anaerobic metabolism in either the exponential phase or the steady phase. The glucose utilized in aerobic metabolism does not exceed 5% of the total consumption of glucose for cell maintenance, which coincides with the reported results[4]. In addition, it is also found that $G_{O/T}$ gradually increases with cell growth. There are two possible reasons. One is that the decrease of glucose concentration in the culture process results in the decline of the EMP flux and the energy from this pathway. Since the energy from the glucose entering the TCA cycle keeps constant, $E_{O/T}$ and $G_{O/T}$ obviously increased in the culture process. It also shows that the maintenance energy from glucose is always descendent in the culture process, and the metabolism of other substances may offset the decrease. Another reason is that cellular metabolism becomes more oxidative at low growth rate[14]. Thus, $E_{O/T}$ and $G_{O/T}$ increase with the decrease of specific growth rate. However, it might explain the data of the steady phase, but is not suitable to those of the lag phase. It is impossible to find the truth by mass balance, and other special experimental techniques such as the method of isotope tracer may be necessary.

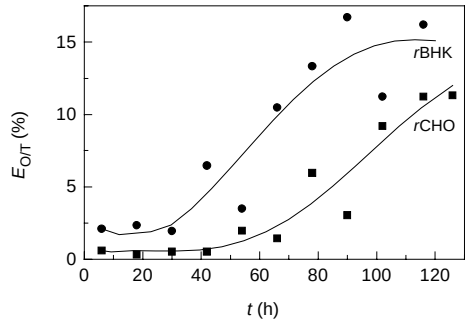

Fig.4 Time profiles of $E_{O/T}$ of *r*CHO and *r*BHK cells

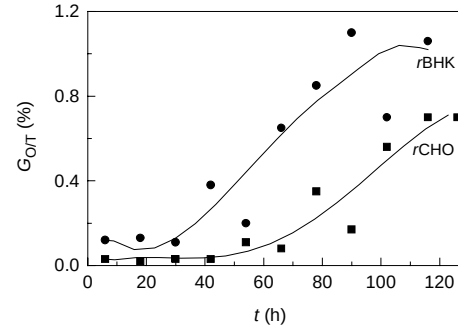

Fig.5 Time profiles of $G_{O/T}$ of *r*CHO and *r*BHK cells

In this paper, the emphasis has been placed on the glucose metabolism and the contribution of its aerobic and anaerobic metabolisms to the maintenance energy. Glutamine can also enter the TCA cycle to provide energy for cell growth and maintenance and can be transformed to lactate[15]. Reitzer et al.[4] found that about 13% of glutamine was transformed to lactate. Because of the low concentration of glutamine, the amount of lactate from glutamine is insignificant in the cell culture. On the other hand, since the maintenance energy supposed here did not include the part from glutamine, the glutamine metabolism exerts negligible effect on the model.

NOMENCLATURE：

A_F	ATP output from anaerobic metabolism of glucose (mmol/L)
A_O	ATP output from aerobic metabolism of glucose (mmol/L)
$E_{O/T}$	Ratio of energy from aerobic glucose metabolism to total energy from glucose metabolism (%)
G	Glucose concentration (mmol/L)
G_F	Glucose concentration utilized by anaerobic metabolic (mmol/L)
G_O	Glucose concentration utilized by aerobic metabolic (mmol/L)
$G_{O/T}$	Ratio of glucose utilized by aerobic metabolism to total amount of cell maintenance (%)
m	Maintenance coefficient [mmol/(cell·h)]

m_O	Maintenance coefficient of aerobic metabolism [mmol/(cell·h)]
P	Product concentration (mg/L)
P_{Ala}	Alanine concentration (mmol/L)
P_{Lac}	Lactate concentration (mmol/L)
t	Time (h)
X	Viable cell density (cell/ml)
Y_G	Yield of cell to glucose (cell/mmol)
Y_p	Yield of product to glucose (mg/mmol)
μ	Apparent specific growth rate (h^{-1})

Subscript

n	n-th sample

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