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Review

Process and metabolic engineering perspectives of lactate production in mammalian cell cultures

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Graphical abstract

Abstract

Mammalian cells present the main expression platforms for production of recombinant therapeutic proteins. To cope with the increased demand for these therapeutics, more productive manufacturing processes have been developed using high-density cultures and enriched feeds/media. This has dramatically increased the productivity of mammalian cells in culture but this is accompanied by an increased production and accumulation of lactate in cultures, with the pattern of phasic production and consumption of lactate associated with the cell productivity in culture. Although primarily defined as a waste product, it is clear that lactate metabolism presents a control node for determination of process control and effectiveness of manufacturing strategies. This review focuses on recent understanding of the phasic nature of lactate metabolism, the impact of culture environment (media, feeds) on lactate metabolism, the link between lactate metabolic status and cell status and the culture/metabolic engineering approaches that have been applied to generate the lactate metabolic phenotype associated with a highly productive manufacturing process

Introduction

In the last 30 years, major improvements in final product titres and viable cell density have been achieved in mammalian cell-based cultures with the upstream process optimisation (i.e., culture operation and tailor-made medium/feeds) [1]. Associated with the drive towards enhanced biomass, metabolic processing of enriched feeds has led to the accumulation of metabolic “waste” products that have consequences on the productivity and quality of therapeutic recombinant (r-) protein products. Great attention has been directed on production and accumulation of lactate in culture as a waste product but recent attention has illustrated that other metabolites can accumulate and become growth limiting to Chinese Hamster Ovary (CHO) cells [2,3]. A recent review has focused on the molecular mechanisms through which lactate metabolism can be regulated [4**]. Here we direct attention to the manufacturing perspective and how lactate metabolism, and its potential as an indicator of process status, offers a model for understanding how it may prove possible to rationalise manufacture optimisation.

In CHO cell systems, lactate concentrations above 20 mM diminish r-protein yield, whilst concentrations exceeding 40 mM severely impair growth [5,6]. The implementation of large-scale fed-batch or perfusion operation using CHO cells has driven lactate accumulation above 80 mM [7,8**,9] and maintenance of the optimal culture pH in bioreactors necessitates addition of high amounts of alkali. Addition of alkali, by increased osmolality, can inhibit cell growth and, additionally, lead to lower r-protein production [10]. A multivariate analysis of Genentech’s manufacturing data highlighted lactate metabolism as a key parameter in determining final productivity of a process [11]. Similar conclusions have been generated by two other independent analyses with data from different manufacturing processes [12,13]. Hence, from a bioprocess perspective, restricting lactate production would aid the development of robust and productive therapeutic r-protein production and, at process and metabolic engineering levels, several strategies have been applied to reduce lactate formation and redirect lactate towards consumption (Figure 1).

Changing lactate metabolism through modification of culture environment

Recent studies exploring the carbon flux distribution in industrially-relevant mammalian cell lines showed that around 60-80% of glucose and 16-25% of glutamine are converted into lactate during the exponential phase [12,13,14,15]. The metabolism status of glucose and glutamine are a key determinant in the switch from lactate production to consumption, an event widely reported to occur when glucose or glutamine availability is limited [5,16,17]. A survey of several manufacturing runs at Genentech indicated that lactate consumption was a prominent factor in determining the productivity of r-proteins in production-scale cultures [11]. The profile of lactate metabolism is not solely down to glucose availability as lactate consumption has been reported even when there is appreciable amount of glucose in the culture medium [16–18]. Two independent studies observed that glutamine availability modulates lactate fate (either production or consumption) at the end of culture [16,18] and a strong positive correlation was observed between the lactate and glutamine metabolism (after the metabolic shift) in CHO cells [18]. As the pattern of lactate metabolism is closely linked to glucose and glutamine metabolism, many of the strategies to develop a decreased lactate production phenotype are focused on the control of these two carbon sources.

Many different sugars, such as galactose, fructose, mannose or maltose, have been proposed as substitutes to glucose, offering the advantage of slower consumption than glucose in culture, leading to less lactate production. Substitution of glucose by galactose, for example, produced significantly less lactate but decreased specific cell growth rate and peak viable cell density [19]. To enhance cell biomass, a biphasic culture strategy (alternating the consumption of glucose and galactose) has been proposed in CHO cell batch and fed-batch cultures [20,21]. Using this strategy, high cell densities have been achieved with significantly decreased (up 66%) lactate accumulation and improved galactosylation in fed-batch cultures [20–22]. Alternatives to glutamine (glutamate, pyruvate, TCA cycle intermediaries and dipeptides) have also been developed. Initially aimed at decreasing ammonia accumulation, these approaches have also impacted on the lactate formation. The use of glutamate, pyruvate or dipeptides such as L-alanyl-L-glutamine (commercially available as

GlutaMAX™) has proven to be reliable substitutes for glutamine and led to decreased lactate accumulation [19,23,24*,25,26]. However, the replacement of glucose and glutamine eventually entails a large impact on glycosylation of therapeutic r-proteins [27,28]. Other medium additives can promote changes to lactate metabolism, although the mechanisms may not be clear. For example, the addition of copper promotes cell growth and r-protein production and decreases lactate formation in several mammalian cell lines [29–31]. However, its use often leads to unpredictable r-protein quality [30,31].

Medium and feed design has been engineered to push the metabolic balance towards the lactate consumption phenotype. Li et al. designed a lactate containing feed to supply energy to cells whilst controlling culture pH [32]. Freund and Croughan (2018) recently reported a Lactate Supplementation and Adaptation (LSA) technology in which CHO cells were adapted to a lactate-supplemented medium which resulted in a near-minimal production of lactate [8**]. When applied to industrial fed-batch bioreactor cultures, LSA led to an 8-fold decrease in lactate production and a high viable cell concentration (around 35 million cells per mL).

Changing lactate metabolism through modification of process

Dynamic feeding approaches have been used to maintain glucose and glutamine at very low concentrations to decrease lactate accumulation in industrial mammalian cell cultures [5,7,29,33]. Application of stoichiometric models or on-line oxygen uptake rate measurement have resulted in considerable decreased lactate accumulation and increased maximal viable cell density and final titre. Although successfully applied in laboratory-scale bioreactors in academia and industry with considerably decreased lactate accumulation in culture, there is no public information of implementation in large-scale mammalian cell cultures. Gagnon et al. proposed a pH-controlled feeding strategy called HI-end pH-controlled Delivery of Glucose (HiPDOG) [7]. Through continuous monitoring and control of residual glucose concentrations, lactate production was substantially decreased with increased product titres in mammalian cell cultures without the use of an external sensor system or frequent sample withdraw. To address the potential problem of failure to feedback control, Goldrick et al designed a highly robust and flexible method to maintain glucose at an adjustable set-point

without the need of an external sensor [34*]. This method has been successfully applied to varied scales (from 15 mL shake flasks to 50 L bioreactors) and cell lines with significantly varied glucose uptake rates. With the implementation of this methodology, cells underwent a lactate switch and depleted almost 100% of lactate produced during the lactate production phase.

Linear correlations have been observed between specific lactate production rate and the setpoints for pH and temperature in batch cultures [35]. Temperature decrease slows cell metabolism, with a decreased consumption of glucose and glutamine and production of lactate both in batch and fed-batch cultures [36,37]. In some studies, decreased culture temperature leads to lactate consumption [17,18], in a cell line-dependent manner. Decreased culture pH, via changes to the activity of glycolytic enzymes and membrane potential, is associated with decreased rates of specific glucose consumption and lactate production [38]. Several studies have shown that the metabolic switch from lactate production to lactate consumption in mammalian cells is dependent on the culture pH setpoint [39,40]. However, as both strategies influence cell growth, r-protein productivity and glycosylation adversely [41,42], the lactate metabolism are optimized on a case-by-case basis in biphasic cultures where these parameters are first controlled to setpoints which favor extensive cell proliferation prior to shifts to setpoints where lactate metabolism is modified [40].

Changing lactate metabolism by metabolic engineering

Regulating the expression of GLUT transporters in combination with the use of alternative carbon sources have been proposed to improve culture performance of recombinant mammalian cells. The most successful case has been the overexpression of the GLUT5 fructose-specific transporter with fructose as main carbon source in culture medium. This strategy led to a dramatic decrease in lactate production rates and an increase in viable cell density compared to glucose-based cultures, in human myeloma derived and CHO cell lines [43,44]. However, the consequence of overexpression of GLUT5 for lactate metabolism by recombinant cell lines depends to a large extent on the regulation of expression of the *glut5* gene. To illustrate this, Le et al. [45] proposed a biphasic culture strategy using a GLUT5 dynamic expression system in concert with cell growth. This strategy enabled cells to consume fructose according to cellular needs rather than in excess as typically seen in culture, thus

driving better cell growth, higher product titre and lower lactate production. Conversely, Wilkens and Gerdtsen using a constitutive GLUT5-overexpressing CHO cell line in a conventional batch with fructose as the main carbon source reported increased cell growth and IgG production, but similar lactate production compared to glucose-based cultures [46].

Prevention of activity of lactate dehydrogenase (LDH-A) appears a direct strategy to decrease lactate production. Elimination of LDH-A by gene knock-out is lethal for mammalian cells [47**]. Consequently, efforts have focused on knocking down (rather than knocking-out) LDH-A activity. Approaches, such as homologous recombination [48], antisense mRNA expression technology [49,50] or small interfering RNA (siRNA) [51], have been shown to decrease LDH-A activity to different degrees. Such modification has led to decreased lactate production and improved cell growth in recombinant hybridoma and CHO cell lines. However, decreasing LDH-A activity had mixed results for carbon metabolism and r-protein production. Suppression of LDH-A activity has been combined with modulation of the expression of other proteins that might be associated with improved cellular phenotype. This includes overexpression of the anti-apoptotic protein Bcl-2 [52] and the down-regulation of pyruvate dehydrogenase kinases (PDHKs) (i.e. PDHK1, 2 and 3) [53] in CHO cells. By applying these strategies, cell cultures dramatically decreased lactate accumulation (by up to 90%) and increased r-protein production. The supplementation of CHO cell cultures with dichloroacetate (DCA), an inhibitor of PDHK, decreased lactate production, increased viable cell density and final antibody titre [47**], supporting gene-level approaches that PDHK are relevant metabolic targets to develop cell factories with improved culture performance. Recently, Noh et al downregulated LDH-A expression in host and a recombinant CHO cell lines with the GS selection system [54*]. The downregulation of LDH-A in the recombinant CHO cell line decreased lactate production and improved N-glycosylation. However, this strategy could not be applied to host cell lines because it hampered the selection process of the glutamine synthetase system [54*]. In a novel approach, Fu et al. developed a lactate-tolerant CHO cell line by overexpressing lactate dehydrogenase C (LDH-C) that led to improved cell growth, decreased lactate production and a more robust apoptosis response in CHO cells [6]. Although this strategy has been only tested in a specific CHO cell line, LDH-C

emerges as an interesting engineering approach for generating host cell lines with better r-protein yield and quality.

As an extension to redirection of pyruvate away from lactate formation, the potential consequence of increasing pyruvate oxidation in mitochondria has been examined in mammalian cell cultures. Overexpression of anaplerotic enzymes, such as pyruvate carboxylase (PC) or malate dehydrogenase (MDH) offer the potential to “pull” pyruvate from a lactate production fate. Many reports have shown that overexpression of cytosolic yeast PC (PYC2) effectively decreases lactate production, improves cell viability and leads to a more efficient use of the carbon sources in recombinant HEK293, BHK-21 and CHO cell lines [24*,55–57]. . However, there are mixed results with respect to r-protein production, glucose consumption and the capacity of cells to shift from a lactate-producing to lactate-consuming phenotype. Most recent studies have highlighted the relevance of upstream process development, particularly the influence that clonal variation and culture process have on the outcoming productivity and nutrient consumption levels of PYC2 expressing cell lines.

Further strategies have been developed to screen and isolate clones with desired metabolic features and production capacity. Gupta et al. [58] implemented a dual selection strategy to select clones based on expression of PYC2 mRNA. With this methodology, they isolated clones that attain an extended fed-batch process with very high cell density, high glucose consumption rate, and significantly decreased lactate production. In a different approach, Toussaint et al. [56] generated a PYC2-expressing CHO cell line and isolated 3 clones with high PYC2 protein expression. All isolated clones presented improved cell growth, r-protein production and a decreased lactate accumulation with a clear metabolic shift towards lactate consumption, both in batch and fed-batch with different culture media. In contrast, studies using polyclonal populations of PYC2-expressing CHO cells (with varying expression of PYC2) showed inconsistent results of in terms of cell growth and productivity, despite an evident decrease in lactate production. PYC2 overexpression in HEK293 and CHO cells dramatically impacted upon the improvement of cell metabolism and r-protein production even under controlled bioreactors culture conditions with optimised medium/feeds composition [24*,55–57].

Overexpression of malate dehydrogenase II (MDH II) has been also proposed as a strategy to overcome a TCA cycle bottleneck and improve energy metabolism in CHO cells [46,59]. These two independent reports developed MDH II-overexpressing CHO cell lines with significantly decreased lactate production and decreased the NAD⁺/NADH ratio but with contradictory results on the value to MDH II as a target to enhance cell growth.

Concluding comments and future perspectives

The increased understanding of lactate metabolism has made it possible to develop strategies that decrease lactate accumulation in cultures. Lactate produced during the culture processes of mammalian cells is regarded as a waste product that is detrimental to efficient cell performance and development of the manufacturing platform. Yet this view is arguably too simplistic. Under specific metabolic (or culture phase) conditions cells can use lactate to provide part of the metabolic input that is associated with prolongment of culture or enhanced production of recombinant proteins. From a manufacturing perspective, lactate production (associated with rapid cell growth during exponential culture phase) is valuable if subsequent consumption of lactate can be built into the cell culture process. Combination of process and metabolic engineering offers a promising approach to generate an effective balance of a limited early lactate production and subsequent lactate consumption. For instance, the overexpression of PYC2 combined with a rational feed design has proven to be a successful approach for the development of high-titre fed-batch cultures with a consistently advantageous profile of lactate metabolism in different industrially-relevant mammalian cell lines [56–58]. Selection of clones with a desirable lactate metabolic profile (low-producing/high-consuming phenotype) offers a route to a better overall metabolic phenotype. However, the lactate-consuming phenotype has been reported to be lost due to unknown factors for certain clones, even in validated cGMP processes making commercial products [11], and such a selection criterion may increase the chances of discarding good production clones.

Ultimately, the question remains open: is it possible to develop a universal and reliable method to generate metabolically super-efficient cell lines that produce high titres in culture? In the opinion of these reviewers, metabolic engineering approaches are still unreliable compared to control process

engineering strategies such as those proposed by Gagnon et al [7], Freund and Croughan [8**] and Goldrick et al. [34*] that have realised the potential to gain the benefits of altered lactate production in large-scale manufacturing processes.

The authors declare no conflicts of interest

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Fig 1