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Employing bacterial microcompartment technology to engineer a shell-free enzyme-aggregate for enhanced 1,2-propanediol production in *Escherichia coli*

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ABSTRACT

Bacterial microcompartments (BMCs) enhance the breakdown of metabolites such as 1,2-propanediol (1,2-PD) to propionic acid. The encapsulation of proteins within the BMC is mediated by the presence of targeting sequences. In an attempt to redesign the Pdu BMC into a 1,2-PD synthesising factory using glycerol as the starting material we added N-terminal targeting sequences to glycerol dehydrogenase, dihydroxyacetone kinase, methylglyoxal synthase and 1,2-propanediol oxidoreductase to allow their inclusion into an empty BMC. 1,2-PD producing strains containing the fused enzymes exhibit a 245% increase in product formation in comparison to un-tagged enzymes, irrespective of the presence of BMCs. Tagging of enzymes with targeting peptides results in the formation of dense protein aggregates within the cell that are shown by immuno-labelling to contain the vast majority of tagged proteins. It can therefore be concluded that these protein inclusions are metabolically active and facilitate the significant increase in product formation.

1. Introduction

Metabolic engineering involves the design and redesign of pathways and their deployment in organisms in which they do not naturally exist. This approach allows pathway fluxes, together with substrate and intermediate concentrations, to be manipulated by variation of the network parameters, which can be quantified by metabolic control analysis (Woolston et al., 2013). However, for pathways involving particularly volatile, unstable or toxic intermediates this tactic is likely to prove problematic. To overcome the problem of capricious metabolites nature has evolved a variety of solutions to ensure pathways operate efficiently without a significant build up of pernicious intermediates. In this respect substrate channelling, multienzyme complexes, metabolons and compartmentalisation are all ways in which pathway flux is naturally controlled (Lee et al., 2012).

In bacteria compartmentalisation is mediated through the deployment of bacterial microcompartments (BMCs), which are used to address the problem of unstable or reactive intermediates (Dou et al., 2008; Havemann et al., 2002; Penrod and Roth, 2006; Sampson and Bobik, 2008). BMCs are proteinaceous complexes that are composed of a semi-porous capsid shell that encases a specific metabolic process (Cheng et al., 2008; Frank et al., 2013; Tanaka et al., 2008; Yeates et al., 2008). The widespread dispersal of BMCs in 23 bacterial phyla mediated through horizontal gene transfer suggests that pathway enhancement through employment of these structures provides a strong evolutionary benefit (Axen et al., 2014). There are two broad classes of BMCs, carboxysomes and metabolosomes, which are associated with either anabolic carbon fixation or catabolic carbon utilisation, respectively. Metabolosomes, in particular, appear to operate pathways that involve aldehydes as intermediates. Indeed, a recent bioinformatics analysis of BMC-associated operons revealed that the vast majority of these operons encode for aldehyde and alcohol dehydrogenases (Axen et al., 2014). The best characterised of the metabolosomes are those associated with 1,2-propanediol utilisation (Pdu) and ethanolamine utilisation (Etu), both of which house cobalamin-dependent enzymes and encase pathways that proceed via propanaldehyde and acetaldehyde respectively (Chowdhury et al., 2014). Compelling evidence has been presented that the compartments help protect the cell from toxicity associated with a high aldehyde concentration (Brinsmade et al., 2005; Sampson and Bobik, 2008; Cheng et al., 2011).

The ability to concentrate a specific metabolic pathway into what is essentially a nano-bioreactor, coupled with the capacity to

Abbreviations: BMC, bacterial microcompartment; Pdu, 1,2-propanediol utilisation; 1,2-PD, 1,2-propanediol; D18, First 18 amino acids of PduD; P18, First 18 amino acids of PduP

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sequester toxic pathway intermediates, has brought BMCs to the attention of synthetic biologists who view this as a tractable system that can be redesigned to accommodate new pathways (Chowdhury et al., 2014; Lawrence et al., 2014). Such a system has the potential to be used to enhance the yield of commodity chemicals produced via bacterial fermentation. Significantly, the *Citrobacter freundii* Pdu BMC can be produced as an empty compartment through the coordinated production of only the shell proteins (PduA, B, B′ J, K, N, U) (Parsons et al., 2010). Enzymes can then be targeted so that they are incorporated into the BMC through the fusion of peptide sequences that are found at the N-terminus of proteins such as PduD (D18) and PduP (P18) (Fan et al., 2010, 2011). Furthermore, targeting of the *Zymomonas mobilis* pyruvate decarboxylase and alcohol dehydrogenase resulted in the conversion of the Pdu BMC into an ethanol bioreactor (Lawrence et al., 2014).

Bio-product commodities that have successfully transitioned into the market through biotechnological approaches include 1,3-propanediol, polylactic acid (PLA) and polyhydroxyalkanoate (PHA), which have been used for personal care products, anti-freeze and biodegradable plastics (Adkesson et al., 2011; Jung et al., 2010; Suriyamongkol et al., 2007). Near-term bio-based products, such as 1,4-butanediol, isobutanol and succinic acid are in progress whilst systems are under development for the production of terpenes and itaconic acid (Burk, et al., 2011; Lee et al., 2005; Peralta-Yahyan et al., 2011, 2012; Steiger et al., 2013; Yim et al., 2011). Approaches such as metabolic engineering and synthetic biology are routinely applied in order to make these processes more efficient and cost competitive. In this paper we outline a method that offers the potential for a significant step-change in bio-commodity production through the development of BMC technology.

The production of 1,2-propanediol from glycerol is similarly recognised as a commercially relevant pathway. 1,2-Propanediol is a commodity chemical that is currently used in the production of plasticisers, antifreeze, thermoset plastics and cosmetics with an annual global demand estimated at around 1.36 million tonnes per year with demand expected to increase over the next few years (Clomburg and Gonzalez, 2011). It is, therefore, of great interest to develop a production method that does not rely on a non-renewable resource (Altaras and Cameron, 1999, 2000; Clomburg and Gonzalez, 2011). Glycerol, on the other hand, is readily available as it is produced as a by-product of the biodiesel production process (Marchetti et al., 2007). It has been reported that for every 100 kg of biodiesel produced 10 kg of glycerol is generated (Yazdani and Gonzalez, 2007). The biochemical pathway for the synthesis of 1,2-propanediol from glycerol (Fig. 1) involves the intermediate methylglyoxal, a compound that is highly toxic to cells in sub-millimolar concentrations (Ferguson et al., 1996). The pathway involves four enzymes, glycerol dehydrogenase (GldA), dihydroxyacetone kinase (DhaK), methylglyoxal synthase (MgsA) and 1,2-propanediol oxidoreductase (FucO). Previously it has been shown that a DNA scaffold enhances 1,2-PD production in *Escherichia coli* from glucose with 3 enzymes including MgsA and GldA (Conrado et al., 2012). Here we set out an alternative approach to determine if the proposed pathway for 1,2-propanediol production could be enhanced through compartmentalisation into a BMC. Moreover we present an alternative approach to compartmentalisation that is the aggregation of enzymes into a supramolecular conglomerate.

The aim of the investigation was therefore to set about creating fusion proteins between known Pdu targeting peptides (D18 and P18) and the four 1,2-propanediol producing enzymes in order to allow their targeting to recombinant empty Pdu BMC system. The effect of the targeting peptides on the activity of the different enzymes and their solubility was investigated. The ability of the targeted enzymes to promote 1,2-propanediol synthesis was determined. The strains were analysed by TEM and protein aggregation was found to play an unexpected but key role in enhancing pathway productivity.

2. Materials and methods

2.1. Plasmid construction

Plasmids were constructed to provide each of the genes of interest with a N-terminal hexa-histidine tag with an optional D18 or P18 targeting peptide.

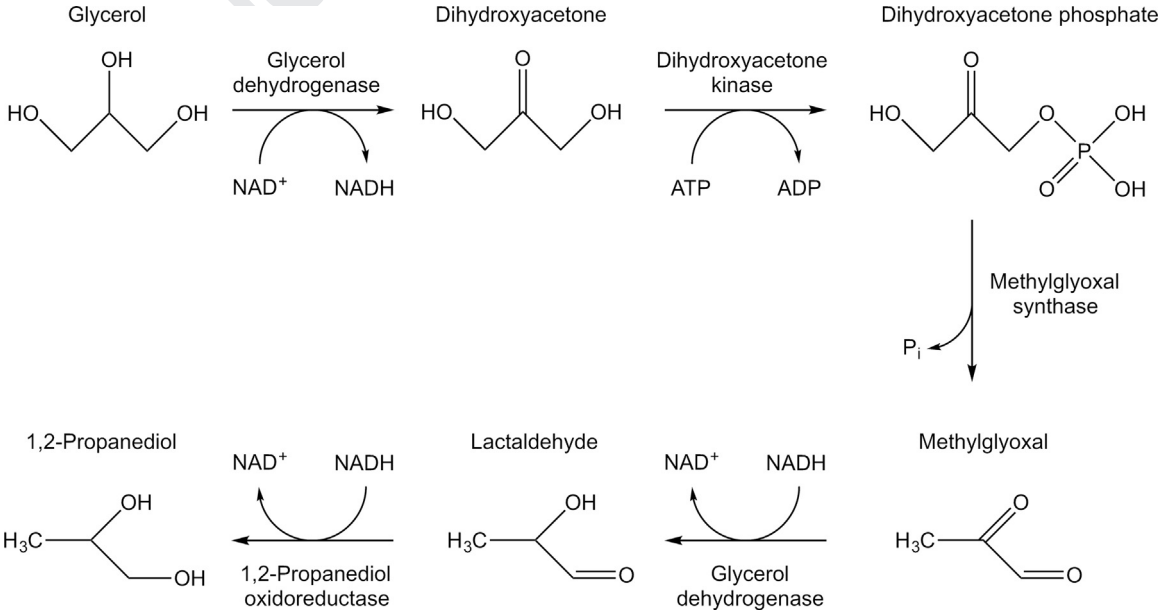

Fig. 1. A pathway for the synthesis of 1,2-propanediol from glycerol. Glycerol dehydrogenase and dihydroxyacetone kinase catalyse the conversion of glycerol to dihydroxyacetone phosphate. Methylglyoxal synthase catalyses the conversion to methylglyoxal. Glycerol dehydrogenase and 1,2-propanediol oxidoreductase catalyse the conversion of methylglyoxal to 1,2-propanediol via the intermediate lactaldehyde.

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