

Review

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Potential use of sugar binding proteins in reactors for regeneration of CO₂ fixation acceptor D-Ribulose-1,5-bisphosphate

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Abstract

Sugar binding proteins and binders of intermediate sugar metabolites derived from microbes are increasingly being used as reagents in new and expanding areas of biotechnology. The fixation of carbon dioxide at emission source has recently emerged as a technology with potentially significant implications for environmental biotechnology. Carbon dioxide is fixed onto a five carbon sugar D-ribulose-1,5-bisphosphate. We present a review of enzymatic and non-enzymatic binding proteins, for 3-phosphoglycerate (3PGA), 3-phosphoglyceraldehyde (3PGAL), dihydroxyacetone phosphate (DHAP), xylulose-5-phosphate (X5P) and ribulose-1,5-bisphosphate (RuBP) which could be potentially used in reactors regenerating RuBP from 3PGA. A series of reactors combined in a linear fashion has been previously shown to convert 3-PGA, (the product of fixed CO₂ on RuBP as starting material) into RuBP (Bhattacharya et al., 2004; Bhattacharya, 2001). This was the basis for designing reactors harboring enzyme complexes/mixtures instead of linear combination of single-enzyme reactors for conversion of 3PGA into RuBP. Specific sugars in such enzyme-complex harboring reactors requires removal at key steps and fed to different reactors necessitating reversible sugar binders. In this review we present an account of existing microbial sugar binding proteins and their potential utility in these operations.

Review

Rapid industrialization has led to a dramatically accelerated consumption of fossil fuels with a consequent increase in atmospheric levels of the greenhouse gas carbon dioxide (CO₂). This sustained increase of atmospheric CO₂ has already initiated a chain of events with negative ecological consequences [1-3]. Failure to reduce these greenhouse gas emissions will have a catastrophic impact upon both the environment and the economy on

a global scale [4,5]. The reduction has to be brought about by global concerted effort by all countries in order to be effective and meaningful.

At one end of the spectrum – that of generation and utilization of energy resulting in generation of carbon dioxide – hydrocarbons serve as intermediaries for energy storage. Hydrocarbons are not energy by themselves but store energy in their bonds, which is released during

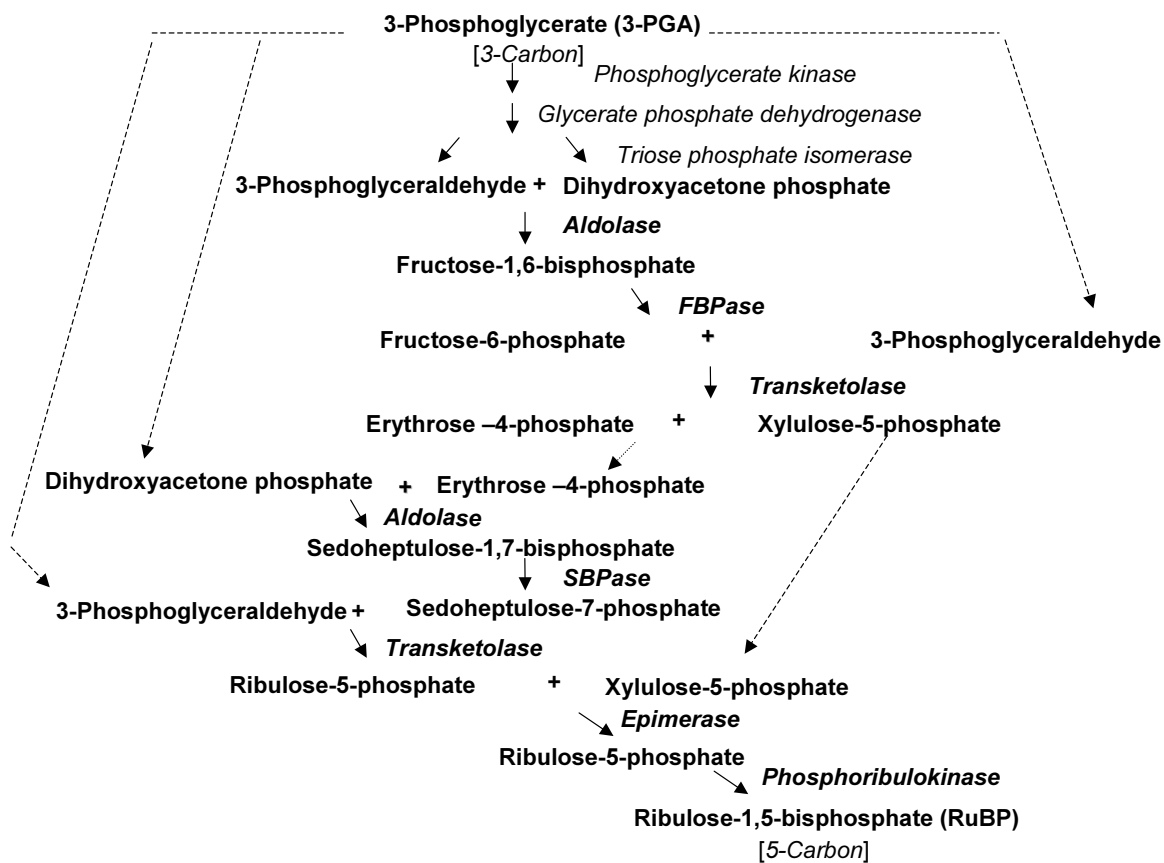


Figure 1

Scheme for generation of D-ribulose-1,5-bisphosphate (RuBP) from 3-phosphoglycerate (3PGA) obtained from fixation of CO₂ on RuBP. The continuous regeneration of RuBP in this scheme enables continuous fixation of CO₂ at stationary emission sites.

combustion. They are thus intermediates for obtaining stored bond energy within them and carbon dioxide is emitted as a consequence of combustion to extract this stored energy. In recent times hydrogen has received renewed attention as the potential replacement for hydrocarbons [6-10]. However, hydrogen too is an intermediary for obtaining stored bond energy. Recent reports suggest that hydrogen as intermediary may not be entirely free from problems. Also, the problems from use of hydrogen as fuel are yet to be fully realized or foreseen [11,12]. In all these endeavors a key question, that whether the hydrocarbons will be still retained as intermediaries in energy utilization and the problem of air pollution caused as a result of their combustion can be technologically

ameliorated, has not been looked in as much detail as perhaps it should have been. This can possibly be achieved by contained handling of carbon dioxide. The contained handling and fixation of CO₂ can be achieved biotechnologically, chemically or by a combination of both.

Sugar binding proteins derived from microbial and other sources have been used for various applications such as diagnostics and affinity purification [13,14], however they have not been used in environmental biotechnological applications. The possibility of their potential application in environmental biotechnology and review of a few potential candidates is presented here.

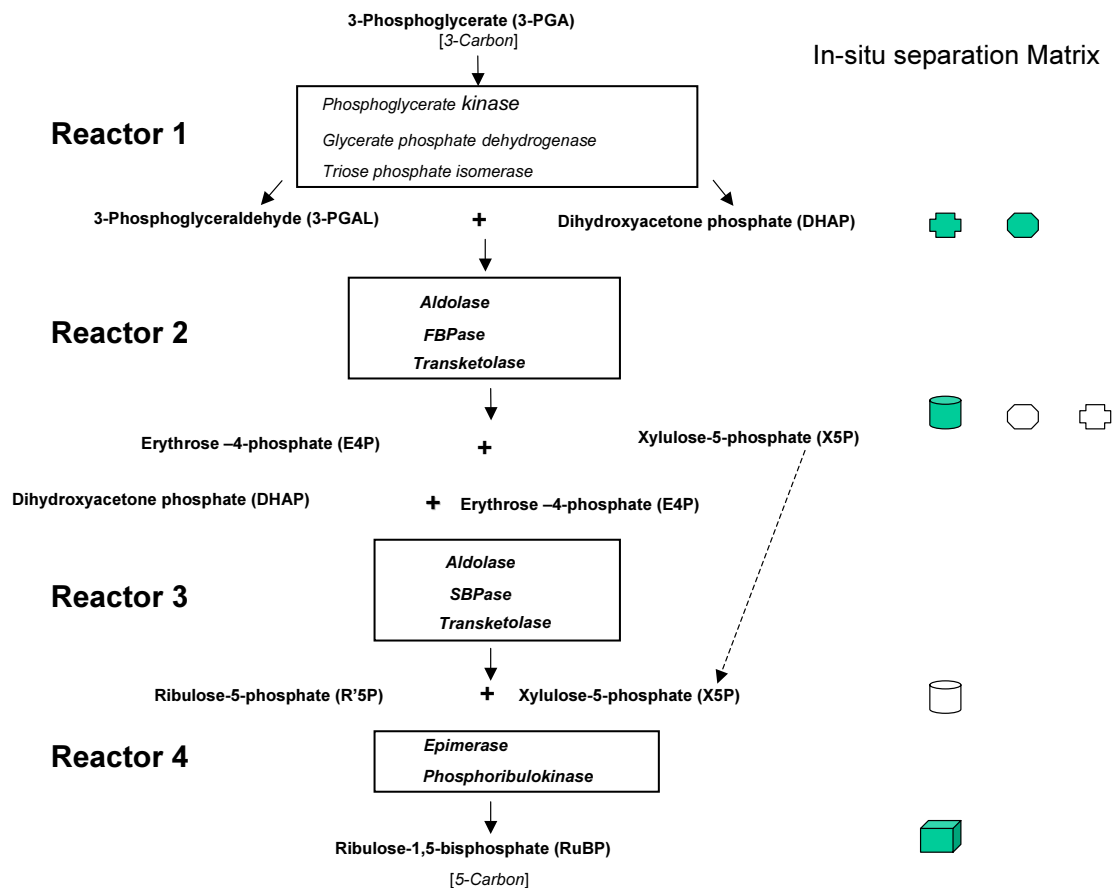


Figure 2

An alternate arrangement of enzymes in the scheme outlined in Fig. 1. This schemes harbors four reactors with indicated enzyme complexes enabling internal channeling, greatly reduces volume and weight for regenerating reactors with faster overall conversion rate to RuBP starting with 3PGA making the system compatible for application in mobile devices in addition to stationary emitters. The reactors may use the sugar binding entities at indicated positions, the hollow and solid symbols represent binding and release phase of the binding-molecules, the plus, circle, cylinder and box are symbols for 3PGA, DHAP, X5P and RuBP binders respectively.

The methods in environmental biotechnology that enables efficient capture [15] and fixation of CO₂ at emission source/site into concatenated carbon compounds has been pioneered by our group [16-19]. The first part in the biocatalytic carbon dioxide fixation is the capture of gaseous CO₂. We have pioneered novel reactors employing immobilized carbonic anhydrase for this purpose [15]. Subsequent to capture the carbon dioxide becomes solubilized (as carbonic acid or bicarbonate). After adjustment of pH using controllers and pH-stat the solution is fed to immobilized Rubisco reactors [18] where acceptor D-Rib-

ulose-1,5-bisphosphate (RuBP) after CO₂ fixation is converted into 3-phosphoglycerate [16,17]. However, inasmuch as the recycling of acceptor RuBP is central to continuous CO₂ fixation, we have invented a novel scheme (Figure 1), which proceeds with no loss of CO₂ (unlike cellular biochemical systems) in 11 steps in a series of bioreactors [20]. This scheme is very different from generation of RuBP from D-glucose for start-up process [21] and employing 11 steps in different reactors requiring large volume and weight. The linear combination of reactors with large volume and weight are unsuit-

Table 1: Proteins that bind 3-phosphoglycerate

| Source | Mutation | Remarks | References |
|---|-----------------------------------|---|------------|
| Enzymatic proteins | | | |
| Phosphoglycerate mutase I (EC 5.4.2.1) | | | |
| <i>E. coli</i> | Glu327 | Lower Vmax | 26 |
| <i>S. cerevisiae</i> | Gly13Ser | 2-fold increase in activity | 27 |
| <i>S. cerevisiae</i> | His181Ala | 11-fold increase in the Km | 28 |
| <i>S. cerevisiae</i> | C-terminal 7 res. Deletion | Loss of activity, retention of ligand binding | 29 |
| <i>B. stearotheophilus</i> | S62A | Loss of activity, retention of ligand binding | 30 |
| <i>S. pombe</i> | H163Q | Reduced mutase and phosphatase activities | 31 |
| <i>E. coli</i> | R257A | 11-fold increase in Vmax | 26 |
| <i>E. coli</i> | R307A | 700-fold decrease in Vmax | 26 |
| Enolase (EC 4.2.1.11) | | | |
| <i>S. cerevisiae</i> | S39A | Loss of over 90% activity | 32 |
| <i>S. cerevisiae</i> | H157A, H159A | Loss of over 90% activity | 33 |
| <i>S. cerevisiae</i> | H159A | Loss of over 98% activity | 34 |
| <i>Escherichia coli</i> | N341D | Loss of catalytic activity | 35 |
| <i>S. cerevisiae</i> | Gcr1-I mutation | 20-fold reduction in activity | 36 |
| Phosphoglycerate kinase, (EC 2.7.2.3) | | | |
| <i>S. cerevisiae</i> | H388G | Reduced kcat and Km | 37 |
| <i>S. cerevisiae</i> | R168K | Increase in Km | 38 |
| <i>S. cerevisiae</i> | R168M | Increase in Km | 38 |
| <i>S. cerevisiae</i> | H62D | Increase in Km and Vmax | 39 |
| <i>S. cerevisiae</i> | D372N | reduction in Vmax by 10-folds | 40 |
| <i>S. cerevisiae</i> | R38A | Complete loss of activity | 41 |
| <i>S. cerevisiae</i> | R38Q | Complete loss of activity | 41 |
| <i>S. cerevisiae</i> | R65Q | Increase in Kd, decrease in Km | 42 |
| <i>S. cerevisiae</i> | R65A | Increase in Kd, decrease in Km | 42 |
| <i>S. cerevisiae</i> | R65S | Increase in Kd, decrease in Km | 42 |
| <i>S. cerevisiae</i> | F194W (and F194L) | decrease in Km, Vmax | 43 |
| <i>S. cerevisiae</i> | R203P | Reduction in kcat | 44 |
| Bisphosphoglycerate mutase (EC 5.4.2.4) | | | |
| <i>S. cerevisiae</i> | H181A | Decrease in kcat | 28 |
| Transketolase | | | |
| <i>S. cerevisiae</i> | E418Q, E418A | 98–99% reduction in activity | 45 |
| <i>S. cerevisiae</i> | E418A | E418 is essential for catalytic activity | 45 |
| <i>S. cerevisiae</i> | H103A, H103N and H103F | 95–99.9% reduced activity | 46 |
| <i>S. cerevisiae</i> | E162A (G) | Impaired catalytic activity and binding | 47 |
| <i>S. cerevisiae</i> | D382N(A) | Impaired catalytic activity and binding | 47 |
| <i>S. cerevisiae</i> | H481A/S/G | 98.5% reduced specific activity | 48 |
| <i>S. cerevisiae</i> | N477A | 1000-fold decrease in kcat/Km | 49 |
| <i>S. cerevisiae</i> | H263A | Reduced activity | 50 |
| D-3-phosphoglycerate dehydrogenase 2 (EC 1.1.1.95) | | | |
| <i>Escherichia coli</i> | L-Serine | Reduced activity | 51 |
| Triosephosphate isomerase | | | |
| <i>Kluyveromyces lactis</i> | KltpiI mutant | Loss of activity | 52 |
| <i>Plasmodium falciparum</i> | Y74G | Reduced stability | 53 |
| <i>Plasmodium falciparum</i> | C13D | 7-fold reduction in activity | 54 |
| <i>Trypanosoma brucei</i> | W12F | Reduced stability | 55 |
| <i>Leishmania mexicana</i> | E65Q | Increased stability | 56 |
| <i>K. lactis</i> | DeltaTPII mutants | Complete loss of activity | 57 |
| <i>Vibrio marinus</i> | A238S mutant | Reduced activity | 58 |
| <i>Trypanosoma brucei</i> | C14L | Reduced stability and altered kinetics | 59 |
| <i>Saccharomyces cerevisiae</i> | K12R | Vmax reduced by factor of 180 | 60 |
| <i>Saccharomyces cerevisiae</i> | K12H | No catalytic activity at neutral pH | 60 |
| <i>Saccharomyces cerevisiae</i> | E165D | 100-fold loss in catalytic activity | 61 |
| <i>Salmonella typhimurium</i> | R179L | Reduction in binding affinity | 62 |
| <i>Trypanosoma brucei</i> | H47N | Reduced stability | 63 |
| <i>Escherichia coli</i> | E165D | 100-fold reduction in specific activity | 64 |
| <i>Escherichia coli</i> | N78D | Lower kcat | 65 |
| <i>Saccharomyces cerevisiae</i> | H95G | 400-fold decrease in catalytic activity | 66 |

Table 1: Proteins that bind 3-phosphoglycerate (Continued)

| Non-enzymatic proteins | |
|--|----|
| Phosphoglycerate transporter protein | |
| <i>Salmonella typhimurium</i> | 67 |
| <i>Salmonella typhimurium</i> | 68 |
| <i>Bacillus cereus</i> | 69 |
| <i>Bacillus anthracis</i> | 70 |
| <i>Salmonella typhi</i> | 71 |
| <i>Salmonella typhi</i> | 72 |
| Histone like DNA-binding protein (HU homolog) | |
| <i>Mycobacterium leprae</i> | 73 |
| <i>Mycobacterium leprae</i> | 74 |
| <i>Mycobacterium tuberculosis</i> | 75 |
| <i>Mycobacterium tuberculosis</i> | 76 |
| 40S ribosomal protein SA (P40) | |
| <i>Chlorohydra viridissima</i> | 77 |
| <i>Strongylocentrotus purpuratus</i> | 78 |
| <i>Tripneustes gratilla</i> | 79 |
| <i>Urechis caupo</i> | 79 |
| Laminin-binding protein | |
| <i>Streptococcus agalactiae</i> | 80 |
| <i>Streptococcus agalactiae</i> | 81 |
| <i>Streptococcus pyogenes</i> | 82 |
| <i>Streptococcus agalactiae</i> | 83 |
| <i>Streptococcus agalactiae</i> | 83 |
| <i>Streptococcus agalactiae</i> | 83 |
| Serine-rich protein (TYE7) | |
| <i>Saccharomyces cerevisiae</i> | 84 |
| <i>Saccharomyces cerevisiae</i> | 85 |

able for use with mobile CO₂ emitters leaving only the stationary source of emission to be controlled using this technology [17]. To circumvent these problems we have devised a new scheme presented in Figure 2 [22]. Based on this scheme, we have designed enzymes as functionally interacting complexes/interactomes or successive conversion in radial flow with layers of uniformly oriented enzymes in concentric circle with axial collection flow system for three enzymes in first reactor for the scheme presented in Figure 2. The four reactors harboring enzymatic complexes/mixtures replace the current 11 reactors. This leads to a faster conversion rate and requires less volume and material weight. However, 4 sugar moieties [3-phosphoglyceraldehyde (3PGAL), Dihydroxyacetone phosphate (DHAP), Xylulose-5-phosphate (X5P) and Ribulose-1, 5-bisphosphate (RuBP)] must be separated at four key steps, as illustrated in Figure 2. In figure 2, using four symbols with solid for bound state and empty for released state, for potential binders: plus for 3PGA, circle for DHAP, cylinder for X5P and box for RuBP, the possible place for utility of these binders have been depicted. In the course of this review, we will consider the availability of enzymatic proteins and non-enzymatic proteins that would be potentially useful as specific binders for these sugar molecules. With a recombinant mutant enzyme we illustrate that such an approach has potential to be used

as an *in-situ* reversible binding matrix for sugar binding and release.

Potential utilizable sugar binding proteins in RuBP regeneration

Three categories of binding proteins can be potentially employed for differential absorption of sugars and for subsequent elution and feeding the reactors downstream in conversion cascade. These are: mutant enzymatic proteins that retain the ability of binding but completely lack any catalytic activity, lectins or proteins of non-immunogenic origin [23] having more than one binding site for the sugar (in nature they cause agglutination of due to sugar binding at multiple sites) and mutant or wild type receptors that binds sugars but are incapable of eliciting further biological activities. The desirable proteins in all these categories are those for which binding affinity is high in a condition close to pH of the emanating solution from the reactor and other conditions for reactor effluent, ability to bind reversibly with respect to some simple but easily manipulable physicochemical parameter (such as temperature, pH, salt concentration), and the ability to be easily attached to a matrix using simple chemistry without loss of binding ability and a long shelf life.

Table 2: Proteins that bind Dihydroxyacetone phosphate

| Source Organism | Mutation | Remarks | References |
|---|-------------------------|--|------------|
| Enzymatic proteins | | | |
| Glyceraldehyde-3-phosphate dehydrogenase | | | |
| <i>S. cerevisiae</i> | ald5 mutant | Higher catalytic activity | 86 |
| <i>S. cerevisiae</i> | gpd2 delta mutant | Improved ethanol production | 87 |
| Dihydroxyacetone kinase I (Glycerone kinase I) | | | |
| <i>Hansenula polymorpha</i> | per6-210 mutant | Lacks enzymatic activity | 88 |
| Glycerol-3-phosphate acyltransferase | | | |
| <i>Escherichia coli</i> | G1045A | Reduced specific activity, increased Km | 89 |
| <i>Escherichia coli</i> | D311E | Reduced catalytic activity | 90 |
| <i>S. cerevisiae</i> | tpa1 mutant | 2-fold decrease in activity | 91 |
| NAD(P)H-dependent dihydroxyacetone-phosphate reductase | | | |
| <i>Escherichia coli</i> | Q15R/K, W37R/K | Inactive with NADP+ | 92 |
| <i>Escherichia coli</i> | Q15K-W37R and Q15R-W37R | 30-fold higher Km for NADP+ | 92 |
| <i>Escherichia coli</i> | gamma-R97Q | 10-fold increased Km for NAD | 93 |
| <i>Escherichia coli</i> | G252A | Reverse transhydrogenation activity | 94 |
| <i>Pseudomonas fluorescens</i> | K295A and K295M | 104–106-fold lower turnover | 95 |
| <i>M. thermoautotrophicum</i> | R11K and R136K | Decreased Km | 96 |
| Alkyl-dihydroxyacetonephosphate synthase | | | |
| <i>Hansenula polymorpha</i> | ts6 and ts44 mutant | Peroxisomes absent | 97 |
| Dihydroxyacetone phosphate acyltransferase | | | |
| <i>Corynebacterium glutamicum</i> | S187C | Reduced enzymatic activity | 98 |
| Triose phosphate isomerase | | | |
| Kltpi1 mutant | | | |
| <i>Kluyveromyces lactis</i> | Y74G | Loss of enzymatic activity | 52 |
| <i>Plasmodium falciparum</i> | C13D | Reduced stability | 53, 54 |
| <i>Plasmodium falciparum</i> | C13D | 7-fold reduction in the enzymatic activity | 53, 54 |
| <i>Trypanosoma brucei</i> | W12F | Reduced stability | 55 |
| <i>Leishmania mexicana</i> | E65Q | Increased stability | 56 |
| <i>K. lactis</i> | | Complete loss of activity | 57 |
| DeltaTPII mutants | | | |
| <i>Bacillus stearothermophilus</i> | N12H | Prevent deamidation at high temperature | 99 |
| <i>Vibrio marinus</i> | A238S | catalytic activity reduced | 58 |
| <i>Trypanosoma brucei</i> | C14L | Reduced stability and altered kinetics | 59 |
| <i>Saccharomyces cerevisiae</i> | K12R | Vmax reduced by a factor of 180, Km elevated | 60 |
| <i>Saccharomyces cerevisiae</i> | K12H | No catalytic activity at neutral pH | 60 |
| <i>Saccharomyces cerevisiae</i> | E165D | 1000-fold reduction in catalytic activity | 61 |
| <i>Salmonella typhimurium</i> | R179L | Reduction in binding affinity | 62 |
| <i>Trypanosoma brucei</i> | H47N | Reduced stability | 63 |
| <i>Escherichia coli</i> | E165D | 1000-fold reduction in specific activity | 64 |
| <i>Escherichia coli</i> | N78D | Lowered Kcat | 65 |
| <i>Saccharomyces cerevisiae</i> | H95G | 400-fold decrease in catalytic activity | 66 |
| Non-enzymatic protein | | | |
| DHAP transporter | | | |
| <i>Saccharomyces cerevisiae</i> | | | 100 |
| <i>mycoplasma mycoides</i> | | | 101 |
| <i>E. coli</i> | | | 102 |
| <i>Pseudomonas aeruginosa</i> | | | 103 |
| <i>Escherichia coli</i> | | | 104 |
| <i>Escherichia coli</i> | | | 105 |
| <i>Escherichia coli</i> | | | 106 |
| <i>Escherichia coli</i> | 107 | | |

We undertook this review because, although the comprehensive information on a large number of enzymes have been accumulated in BRENDA database [24,25], but the systematic information on their mutants is lacking and

non-enzymatic binders of sugar ligands are not identified / listed in the database.

Table 3: Proteins that bind Xylulose-5-phosphate

| Source | Mutation | Remarks | References |
|--|--------------|---------------------------|------------|
| Enzymatic proteins | | | |
| 1-deoxy-D-xylulose 5-phosphate reductoisomerase | | | |
| <i>Escherichia coli</i> | E231K | 0.24% wild-type kcat | 108 |
| <i>Escherichia coli</i> | H153Q | 3.5-fold increase in Km | 108 |
| <i>Escherichia coli</i> | H209Q | 7.6-fold increase in Km | 108 |
| <i>Escherichia coli</i> | H257Q | 19-fold increase in Km | 108 |
| xylulose kinase | | | |
| <i>Escherichia coli</i> | XylB- mutant | Lack of growth on xylitol | 109 |
| Dihydroxyacetone synthase | | | |
| <i>Hansenula polymorpha</i> | Pex1-6(ts) | Peroxisome-deficient | 110 |
| <i>Hansenula polymorpha</i> | Deltapex14 | Lack normal peroxisomes | 111 |
| Non-enzymatic proteins | | | |
| Xylulose-5-phosphate receptor | | | |
| <i>Mycobacterium tuberculosis</i> | | | 112 |
| Xylulose-5-phosphate transporter | | | |
| <i>Arabidopsis</i> sp. | | | 113 |

Proteins that bind 3-phosphoglycerate/3-phosphoglyceraldehyde

Both enzymatic and non-enzymatic proteins bind these sugar entities. A number of mutants of many enzymes that bind to either 3-phosphoglycerate or 3-phosphoglyceraldehyde are also known, for example, Phosphoglyceromutase (EC 5.4.2.1), Enolase (EC 4.2.1.11), Mannosyl-3-phosphoglycerate phosphatase (EC 3.1.3.70), Mannosyl-3-phosphoglycerate synthase (EC 2.4.1.217), Phosphoglycerate kinase, (EC 2.7.2.3), Bisphosphoglycerate mutase (EC 5.4.2.4), 2,3-bisphosphoglycerate-independent phosphoglycerate mutase (EC 5.4.2.1), D-3-phosphoglycerate dehydrogenase 2 (EC 1.1.1.95), Cyclic 2,3-diphosphoglycerate-synthetase, Phosphoglycerate dehydrogenase, Transketolase, and Triosephosphate isomerase, BRENDA database shows only three enzymes: Phosphoglycerate dehydrogenase, Mannosyl-3-phosphoglycerate synthase and Phosphoglycerate kinase. A number of mutants of enzymes that binds 3-phosphoglycerate and shows some change in enzymatic activity or kinetic parameters are listed in Table 1. Many of these proteins are reported to retain ligand binding ability with varying degree of loss in catalytic ability (inactive mutants are in bold face), the non-enzymatic protein that also has been reported in literature has been placed towards the bottom part of Table 1. The proteins which retain binding ability but with complete loss in catalytic activity are the ones which warrant further investigation in batch and continuous processes for exploring their suitability as binding proteins in continuous RuBP regenerating reactors (Figure 2). A number of non-enzymatic protein summarized in Table 1 also warrant further exploration. The only binding entity of significance for 3-

phosphoglyceraldehyde is 3-phosphoglyceraldehyde dehydrogenase (EC 1.2.1.12) and has not been reviewed.

Proteins that bind dihydroxyacetone phosphate

Several enzymes: dihydroxyacetone phosphate acyltransferase, Glycerol-3-phosphate dehydrogenase, Aldolase A, fructose-bisphosphatase, Aldolase B, fructose-bisphosphatase, L-aspartate oxidase, Quinolinic synthetase A, Dihydroxyacetone kinase 1 (Glycerone kinase 1), Glycerol-3-phosphate acyltransferase, NAD(P)H-dependent dihydroxyacetone-phosphate reductase, Dihydroxyacetone phosphate acyltransferase, Alkyl-dihydroxyacetonephosphate synthase, Dihydroxyacetone kinase isoenzyme I, Alpha-glycerophosphate oxidase and Triose phosphate isomerase binds DHAP (Table 2), however, BRENDA shows only four of these proteins, glycerol-3-phosphate dehydrogenase (EC 1.1.1.8), acylglycerone-phosphate reductase (EC 1.1.1.101), glycerone-phosphate O-acyltransferase (EC 2.3.1.42) and alkylglycerone-phosphate synthase (2.5.1.26). The mutants of enzymes with no chemical conversion ability but with high affinity for binding dihydroxyacetone phosphate but very low affinity for other proteins and reversible binding with respect to temperature, salt or pH are desirable properties for the binders.

Proteins binding xylulose-5-phosphate

As shown in Table 3 several enzymatic proteins binds to xylulose-5-phosphate. Xylulose-5-phosphate phosphoketolase, Dihydroxyacetone synthase, xylulose kinase, Protein phosphatase 2A B alpha isoform, Xylulose 5-phosphate-activated protein phosphatase, 1-deoxy-D-xylulose 5-phosphate reductoisomerase, 1-deoxy-D-xylulose 5-phosphate synthase 1 and 2 are examples of such

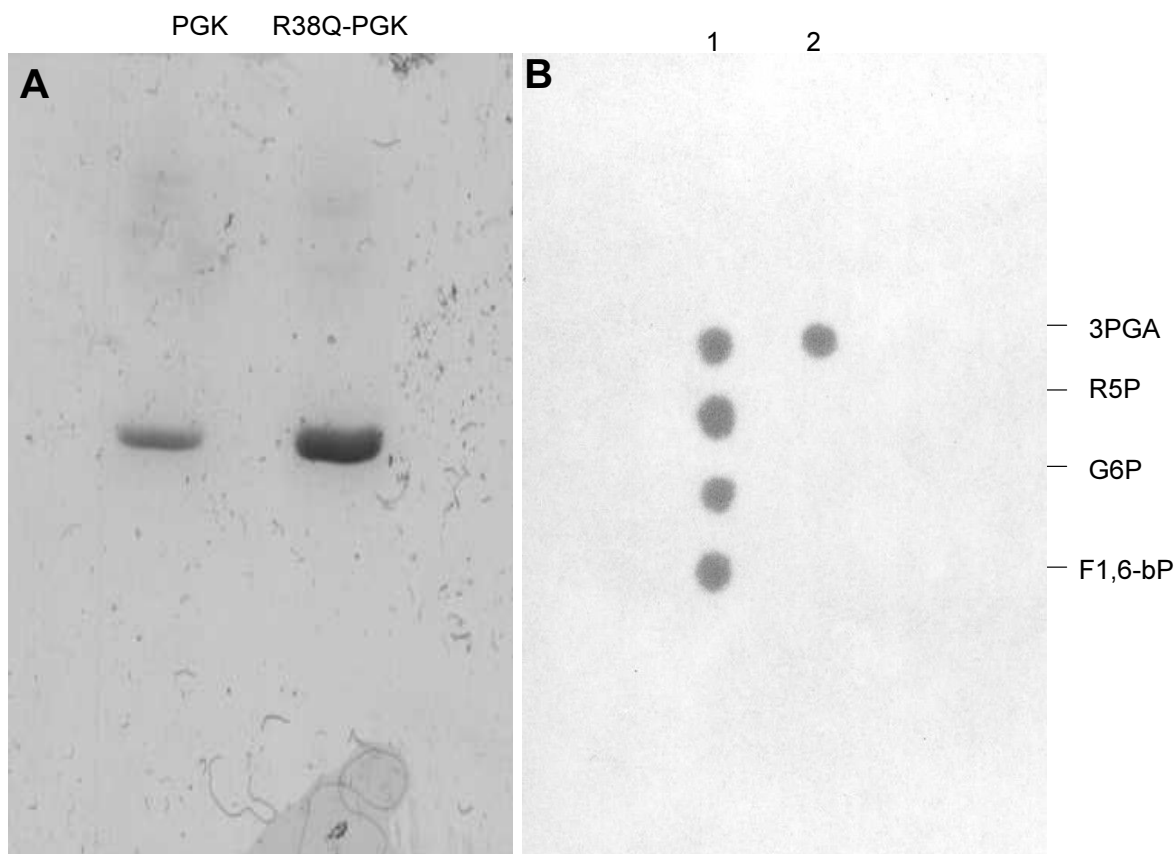


Figure 3

The recombinant his-tagged wild-type and R38Q mutant 3-phosphoglycerate kinase was subjected to affinity purification on Ni-NTA column as described previously [20]. **A.** SDS-PAGE of recombinant wild-type and R38Q mutant *S. cerevisiae* 3-phosphoglycerate kinase. The proteins (1 and 1.8 μ g respectively) was separated in 10% polyacrylamide gel and stained with Coomassie blue R250. **B.** TLC analysis of sugars prior to and after in-situ separation with R38Q. The recombinant R38Q mutant (R38Q-PGK) was coupled with Protein A sepharose beads and incubated overnight with a mixture of sugars, 3-phosphoglycerate (3PGA), ribulose-5-phosphate (R5P), Glucose-6-phosphate (G6P) and Fructose-6-phosphate (F1,6-bP). After washing with 180 mM NaCl, the sugars were eluted with 1 M NaCl. Lane 1, mixture of sugar prior to incubation with R38Q-PGK and Lane 2 after elution with 1 M NaCl.

enzymes. The non-enzymatic xylulose-5-phosphate binders are shown in the bottom part of Table 3. BRENDA database shows following five proteins, 1-deoxy-D-xylulose-5-phosphate reductoisomerase (EC 1.1.1.267), formaldehyde transketolase (EC 2.2.1.3), 1-deoxy-D-xylulose 5-phosphate synthase (EC 2.2.1.7), Phosphoketolase (EC 4.1.2.9), Ribulose-phosphate 3-epimerase (EC 5.1.3.1).

Proteins binding D-Ribulose-1,5-bisphosphate

A number of Ribulose-1,5-bisphosphate and metabolizing enzymes such as Ribulose phosphate kinase and their mutants binds D-ribulose-1,5-bisphosphate. The RuBP binding entities devoid of any enzymatic activities are very valuable in reactors necessitating extraction and separation of RuBP from other sugar compounds (Table 4). Very few non-enzymatic proteins bind RuBP and none of them are microbial sources, and hence have not been

Table 4: Enzymes that bind D-Ribulose-1,5-bisphosphate

| Source organism | Mutation | Remarks | References |
|----------------------------------|---------------------|--|------------|
| Rubisco | | | |
| <i>Chamydomonas reinhardtii</i> | C256F, K258R, L265V | 85% decrease in Catalytic efficiency (V_{max}/K_m) | 114 |
| <i>Chamydomonas reinhardtii</i> | G54V | 83% decrease in the carboxylation- V_{max} | 115 |
| <i>Anacystis nidulans</i> | L339F, A340L, S341M | Decrease in Kcat and (V_{max}/K_m) by 90% and 36.3% respectively | 116 |
| <i>Anacystis nidulans</i> | T342I, K343L | Decrease in Kcat and (V_{max}/K_m) by 90% and 36.3% respectively | 116 |
| <i>Anacystis nidulans</i> | T342I | Decrease in Kcat and (V_{max}/K_m) 40.5% and 40.5% respectively | 116 |
| <i>Anacystis nidulans</i> | K343L | Decrease in Kcat and (V_{max}/K_m) 48.1% and 18.5% respectively | 116 |
| <i>Anacystis nidulans</i> | V346Y, D347H, L348T | Inactive | 116 |
| <i>Anacystis nidulans</i> | L326I | Decrease in Kcat and (V_{max}/K_m) 54.4% and 34.2% respectively | 116 |
| <i>Anacystis nidulans</i> | S328A | Decrease in Kcat and (V_{max}/K_m) 5.6% and 41.5% respectively | 116 |
| <i>Anacystis nidulans</i> | N123H | 16.5% decrease in Kcat | 116 |
| <i>Anacystis nidulans</i> | L332M, L332I | >65% decrease in carboxylase but not in oxygenase activity | 117 |
| <i>Anacystis nidulans</i> | | >65% decrease in carboxylase but not in oxygenase activity | 117 |
| <i>Anacystis nidulans</i> | L332V | 67% decrease in specificity factor (CO_2/O_2) | 117 |
| <i>Anacystis nidulans</i> | L332T | 67% decrease in specificity factor (CO_2/O_2) | 117 |
| <i>Anacystis nidulans</i> | L332A | >65% decrease in specificity and carboxylase activity | 117 |
| <i>Rhodospirillum rubrum</i> | deletion of F327 | 99.5% decrease in carboxylase activity | 118 |
| <i>Rhodospirillum rubrum</i> | F327L | Increase in K_m (RuBP) | 118 |
| <i>Rhodospirillum rubrum</i> | F327V | Increase in K_m (RuBP) | 118 |
| <i>Rhodospirillum rubrum</i> | F327A | Increase in K_m (RuBP) | 118 |
| <i>Rhodospirillum rubrum</i> | F327G | 165-fold increase in K_m (RuBP) | 118 |
| <i>Rhodospirillum rubrum</i> | N111G | K_m (RuBP), kcat are 320 fold increased and 88-fold decreased | 119 |
| <i>Rhodospirillum rubrum</i> | N111L | Mutant show a very low carboxylase activity | 119 |
| <i>Rhodospirillum rubrum</i> | N111Q | Mutant show a very low carboxylase activity | 119 |
| <i>Rhodospirillum rubrum</i> | N111B | Mutant show a very low carboxylase activity | 119 |
| <i>Synechococcus</i> sp.PCC6301 | I87V | Mutant show a very low carboxylase activity (kcat = 35%) | 120 |
| <i>Synechococcus</i> sp.PCC6301 | R88K | Mutant show a very low carboxylase activity (kcat = 35%) | 120 |
| <i>Synechococcus</i> sp.PCC6301 | G91V | Mutant show a very low carboxylase activity (kcat = 35%) | 120 |
| <i>Synechococcus</i> sp.PCC6301 | F92L | Mutant show a very low carboxylase activity (kcat = 35%) | 120 |
| <i>Synechococcus</i> sp.PCC6803 | C172A | 40–60% decline in Rubisco turnover number | 121 |
| <i>Chlamydomonas reinhardtii</i> | N123G | Decrease in specificity factor | 122 |
| <i>Chlamydomonas reinhardtii</i> | S379A | Decrease in specificity factor | 122 |
| <i>Anacystis nidulans</i> | S376 C | 99% and ~99.9% decrease in carboxylase and oxygenase activity | 123 |
| <i>Anacystis nidulans</i> | S376 T | 99% and ~99.9% decrease in carboxylase and oxygenase activity | 123 |
| <i>Anacystis nidulans</i> | S376 A | 99% and ~16% decrease in carboxylase and oxygenase activity | 123 |
| <i>Rhodospirillum rubrum</i> | I164T | 6% decrease in carboxylase activity with 40-fold lower Kcat/ K_m | 124 |
| <i>Rhodospirillum rubrum</i> | I164N | 1% decrease in carboxylase activity with 900-fold lower Kcat/ K_m | 124 |
| <i>Rhodospirillum rubrum</i> | I164B | 0.01–1% decrease in carboxylase activity | 124 |
| <i>Rhodospirillum rubrum</i> | H287N | 10^3 -fold decrease in carboxylation catalysis | 125 |
| <i>Rhodospirillum rubrum</i> | H287Q | 10^5 -fold decrease in carboxylation catalysis | 125 |
| <i>Rhodospirillum rubrum</i> | M330L | | 126 |
| Rubisco (large subunit) | | | |
| <i>Chamydomonas reinhardtii</i> | R59A | Decrease in V_{max} for carboxylation reaction | 127 |
| <i>Chamydomonas reinhardtii</i> | Y67A | Decrease in V_{max} for carboxylation reaction | 127 |
| <i>Chamydomonas reinhardtii</i> | Y68A | Decrease in V_{max} for carboxylation reaction | 127 |
| <i>Chamydomonas reinhardtii</i> | D69A | Decrease in V_{max} for carboxylation reaction | 127 |
| <i>Chamydomonas reinhardtii</i> | R71A | decrease in V_{max} (for carboxylation reaction) and thermal stability | 127 |
| <i>Chamydomonas reinhardtii</i> | A222T, V262L, L290F | Improved specificity factor and thermal stability | 128 |
| Phosphoribulokinase | | | |
| <i>Rhodobacter sphaeroides</i> | T18A | 8-fold decrease in V_{max} | 129 |
| <i>Rhodobacter sphaeroides</i> | S14A | 40-fold decrease in V_{max} | 129 |
| <i>Rhodobacter sphaeroides</i> | S19A | 500-fold and >1500-fold decrease in V_{max} and V_{max}/K_m of RuBP | 129 |
| <i>Rhodobacter sphaeroides</i> | K165M, K165C | 10^3 -fold decrease in catalytic activity | 130 |
| <i>Rhodobacter sphaeroides</i> | R168Q | >300-fold decrease in catalytic efficiency | 131 |
| <i>Rhodobacter sphaeroides</i> | R173Q | 15-fold decrease in V_{max} , 100-fold increase in K_m for RuBP | 131 |

Table 4: Enzymes that bind D-Ribulose-1,5-bisphosphate (Continued)

| | | | |
|----------------------------------|-------------|--|-----|
| <i>Chlamydomonas reinhardtii</i> | R64C | Almost inactive | 132 |
| <i>Chlamydomonas reinhardtii</i> | R64A | Decrease in activity | 132 |
| <i>Chlamydomonas reinhardtii</i> | R64K | Decrease in activity | 132 |
| <i>Synechocystis</i> sp. | S222F | Retains one-tenth catalytic activity | 133 |
| <i>Rhodobacter sphaeroides</i> | H45N | 40-fold increase in Km for RuBP | 134 |
| <i>Rhodobacter sphaeroides</i> | N49Q | 200-fold increase in Km for RuBP | 134 |
| <i>Rhodobacter sphaeroides</i> | K53M | No effect on catalysis or substrate binding | 134 |
| <i>Rhodobacter sphaeroides</i> | D169A | V_{max} diminished by 4-orders of magnitude | 135 |
| <i>Rhodobacter sphaeroides</i> | D42A | V_{max} diminished by 5-orders of magnitude | 135 |
| <i>Rhodobacter sphaeroides</i> | D42N | V_{max} diminished by 5-orders of magnitude | 135 |
| <i>Rhodobacter sphaeroides</i> | R31A | Unlike wild-type, shows hyperbolic kinetics for ATP and NADH | 136 |

incorporated in this review, Rubisco associated protein from soybean is one of them, that show significant RuBP binding [137].

Illustrating example

In order to illustrate the utility of non-catalytic enzymatic mutants as specific sugar binders for in-situ separation in reactors, recombinant *Saccharomyces cerevisiae* 3-phosphoglycerate kinase mutant R38Q [41] was prepared. Mutagenesis was carried out using wild type protein construct in plasmid pET19b as a template. The R38Q mutant was constructed with the Quickchange/Chameleon site-directed mutagenesis kit from stratagene using primers as described elsewhere [41]. DNA sequencing of the plasmid identified the mutant. Recombinant wild-type and mutant (R38Q) 3-phosphoglycerate kinase (PGK) were purified to apparent homogeneity as described previously [20] have been shown in Figure 3A. The wild-type and mutant protein was incubated with 10 mM 3-phosphoglycerate barium salt (3PGA) in 50 mM Tris-Cl buffer, pH 7.5 containing 50 mM NaCl for overnight at room temperature. No modification of 3PGA was observed after incubation with R38Q mutant protein (data not shown). The R38Q was coupled with Protein A sepharose beads using dimethylpimelimidate. The recombinant R38Q mutant protein beads (R38Q-PGK) was incubated overnight at room temperature with a mixture of sugars, 3-phosphoglycerate, barium salt (3PGA), ribulose-5-phosphate (R5P), Glucose-6-phosphate (G6P) and Fructose-6-phosphate (F1,6-bP) each at a concentration of 10 mM in a volume of 200 μ l. After incubation they were washed with 1.5 ml of 180 mM NaCl in 50 mM Tris-Cl buffer, pH 7.5. They were subjected to elution with 1 M NaCl. Lane 1, mixture of sugar prior to incubation with R38Q-PGK and Lane-2 after elution with 1 M NaCl.

Conclusion

The enzyme-mutants lacking catalytic activity represent an important group of proteins that could be used for development of sugar-binding proteins reversible with respect

to physicochemical parameters such as pH or salt concentration. Nevertheless, the non-enzymatic proteins also represent a suitable repertoire of such potential scaffolds, which could be used for development as sugar-binding proteins to be used in reactors for simultaneous separation of sugars that would be used in subsequent conversion steps. We have developed a RuBP production scheme from 3PGA [16,17] and also a *de novo* RuBP production scheme from D-glucose [21] for continuous CO₂ fixation and for start-up of the fixation respectively employing series of reactors. Both systems for production of RuBP will benefit from specific sugar binders but besides their use in environmental biotechnology, they will find application in diagnostics, separation technologies and also as research reagents.

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