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Review

Potential use of sugar binding proteins in reactors for regeneration of CO_2 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 CO_2 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 (CO_2). This sustained increase of atmospheric CO_2 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

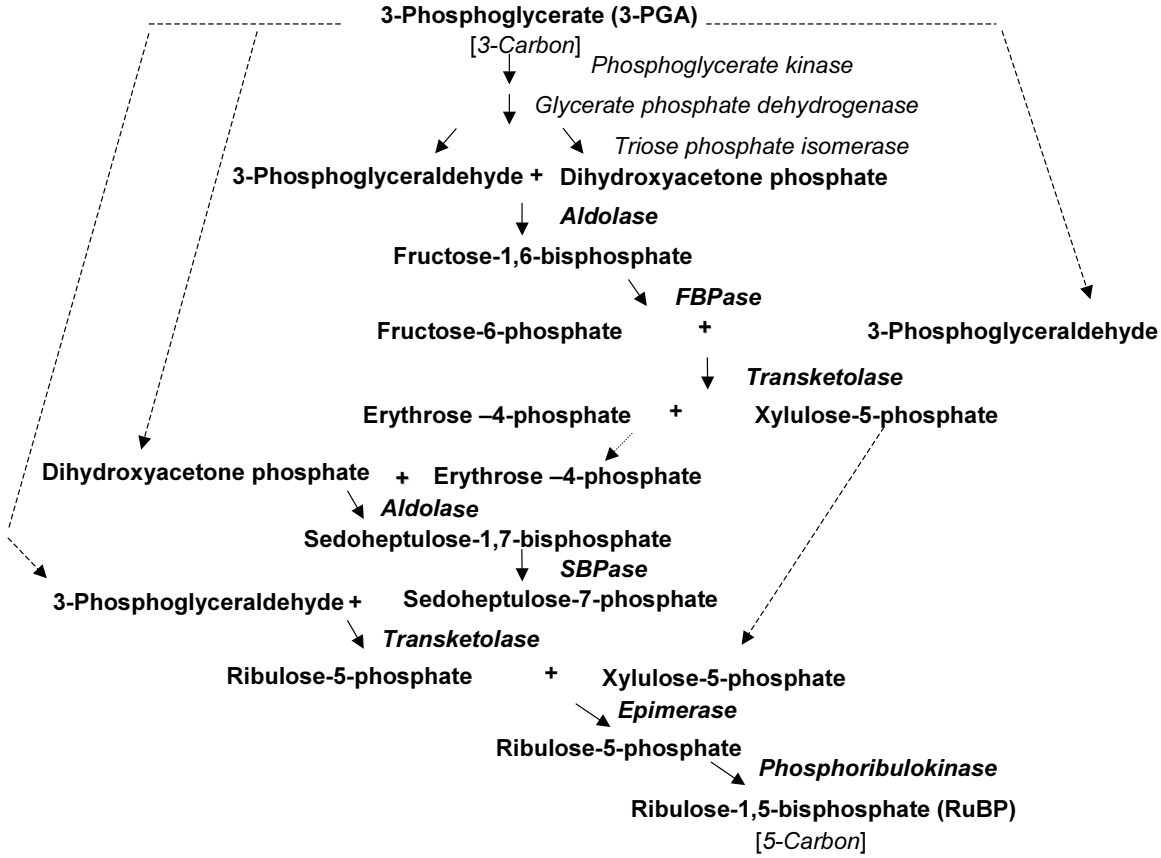

Figure 1
Scheme for generation of D-ribulose-1,5-bisphosphate (RuBP) from 3-phosphoglycerate (3PGA) obtained from fixation of CO_2 on RuBP. The continuous regeneration of RuBP in this scheme enables continuous fixation of CO_2 at stationary emission sites.

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 CO_2 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.

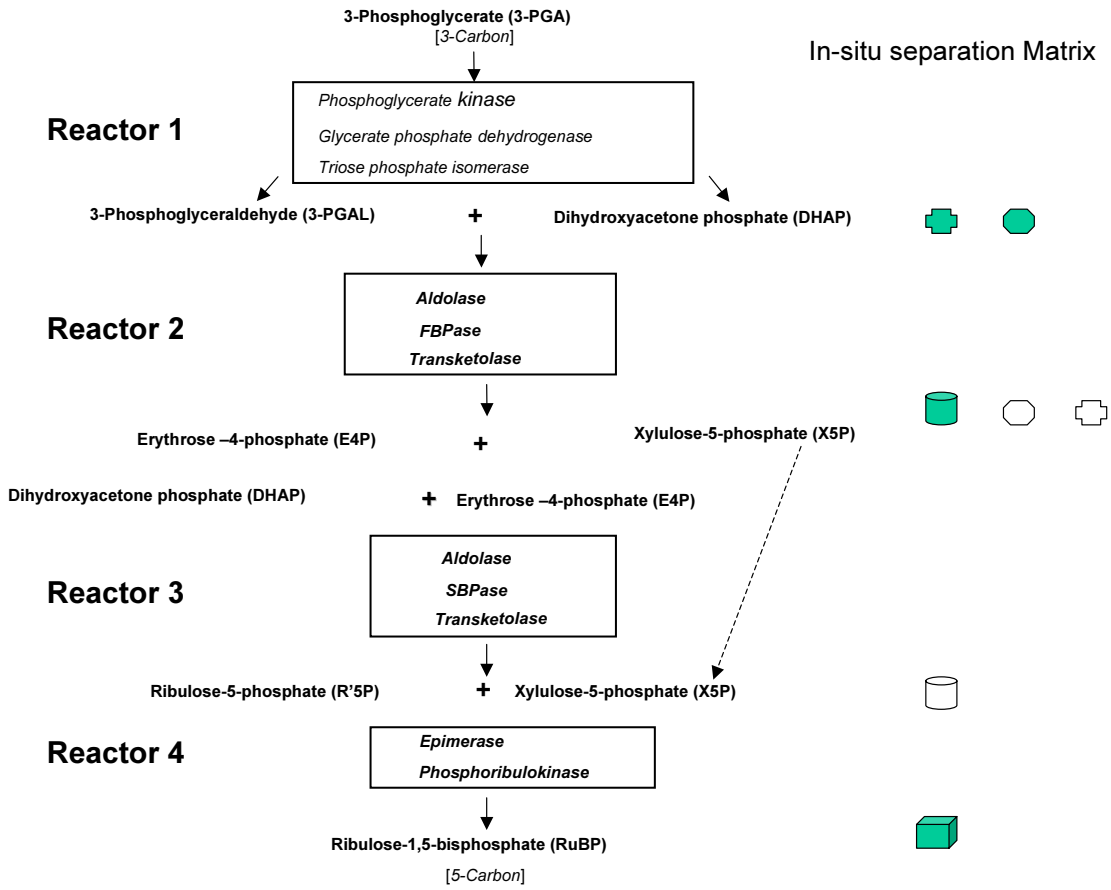

Figure 2
An alternate arrangement of enzymes in the scheme outlined in Fig. 1. This schemes harbors four reactors with indicated enzyme complexes enabling internal channeling, greatly reduces volume and weight for regenerating reactors with faster overall conversion rate to RuBP starting with 3PGA making 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 CO_2 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 CO_2. We have pioneered novel reactors employing immobilized carbonic anhydrase for this purpose [15]. Subsequent to capture the carbon dioxide becomes solublized (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-Ribulose-1,5-bisphosphate (RuBP) after CO_2 fixation is converted into 3-phosphoglycerate [16,17]. However, inasmuch as the recycling of acceptor RuBP is central to continuous CO_2 fixation, we have invented a novel scheme (Figure 1), which proceeds with no loss of CO_2 (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 1 (EC 5.4.2.1)		
E. coli	Glu327	Lower Vmax	26
S. cerevisiae	Gly13Ser	2-fold increase in activity	27
S. cerevisiae	His181Ala	11-fold increase in the Km	28
S. cerevisiae	**C-terminal 7 res. Deletion**	Loss of activity, retention of ligand binding	29
B. stearothermophilus	**S62A**	Loss of activity, retention of ligand binding	30
S. pombe	H163Q	Reduced mutase and phosphatase activities	31
E. coli	R257A	11-fold increase in Vmax	26
E. coli	R307A	700-fold decrease in Vmax	26
	Enolase (EC 4.2.1.11)		
S. cerrevisiae	**S39A**	Loss of over 90% activity	32
S. cerrevisiae	**H157A, H159A**	Loss of over 90% activity	33
S. cerrevisiae	**H159A**	Loss of over 98% activity	34
Escherichia coli	**N341D**	Loss of catalytic activity	35
S. cerrevisiae	Gcr1-1 mutation	20-fold reduction in activity	36
	Phosphoglycerate kinase, (EC 2.7.2.3)		
S. cerrevisiae	H388G	Reduced kcat and Km	37
S. cerrevisiae	R168K	Increase in Km	38
S. cerrevisiae	R168M	Increase in Km	38
S. cerrevisiae	H62D	Increase in Km and Vmax	39
S. cerrevisiae	D372N	reduction in Vmax by 10-folds	40
S. cerrevisiae	**R38A**	Complete loss of activity	41
S. cerrevisiae	**R38Q**	Complete loss of activity	41
S. cerrevisiae	R65Q	Increase in Kd, decrease in Km	42
S. cerrevisiae	R65A	Increase in Kd, decrease in Km	42
S. cerrevisiae	R65S	Increase in Kd, decrease in Km	42
S. cerrevisiae	F194W (and F194L)	decrease in Km, Vmax	43
S. cerrevisiae	R203P	Reduction in kcat	44
	Bisphosphoglycerate mutase (EC 5.4.2.4)		
S. cerevisiae	H181A	Decrease in kcat	28
	Transketolase		
S. cerevisiae	**E418Q, E418A**	98–99% reduction in activity	45
S. cerevisiae	E418A	E418 is essential for catalytic activity	45
S. cerevisiae	H103A, H103N and H103F	95–99.9% reduced activity	46
S. cerevisiae	E162A (G)	Impaired catalytic activity and binding	47
S. cerevisiae	D382N(A)	Impaired catalytic activity and binding	47
S. cerevisiae	H481A/S/G	98.5% reduced specific activity	48
S. cerevisiae	N477A	1000-fold decrease in kcat/Km	49
S. cerevisiae	H263A	Reduced activity	50
	D-3-phosphoglycerate dehydrogenase 2 (EC 1.1.1.95)		
Escherichia coli	L-Serine	Reduced activity	51
	Triosephosphate isomerase		
Kluyveromyces lactis	**Kltpi1 mutant**	Loss of activity	52
Plasmodium falciparum	Y74G	Reduced stability	53
Plasmodium falciparum	C13D	7-fold reduction in activity	54
Trypanosoma brucei	W12F	Reduced stability	55
Leishmania mexicana	E65Q	Increased stability	56
K. lactis	**DeltaTPI1 mutants**	Complete loss of activity	57
Vibrio marinus	A238S mutant	Reduced activity	58
Trypanosoma brucei	C14L	Reduced stability and altered kinetics	59
Saccharomyces cerevisiae	K12R	Vmax reduced by factor of 180	60
Saccharomyces cerevisiae	K12H	No catalytic activity at neutral pH	60
Saccharomyces cerevisiae	E165D	100-fold loss in catalytic activity	61
Salmonella typhimurium	R179L	Reduction in binding affinity	62
Trypanosoma brucei	H47N	Reduced stability	63
Escherechia coli	E165D	100-fold reduction in specific activity	64
Escherechia coli	N78D	Lower kcat	65
Saccharomyces cerevisiae	H95G	400-fold decrease in catalytic activity	66

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

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

able for use with mobile CO_2 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		
S. cerevisiae	ald5 mutant	Higher catalytic activity	86
S. cerevisiae	gpd2 delta mutant	Improved ethanol production	87
	Dihydroxyacetone kinase 1 (Glycerone kinase 1)		
Hansenula polymorpha	**per6-210 mutant**	Lacks enzymatic activity	88
	Glycerol-3-phosphate acyltransferase		
Escherichia coli	G1045A	Reduced specific activity, increased Km	89
Escherichia coli	D311E	Reduced catalytic activity	90
S. cerevisiae	tpa1 mutant	2-fold decrease in activity	91
	NAD(P)H-dependent dihydroxyacetone-phosphate reductase		
Escherichia coli	Q15R/K, W37R/K	Inactive with NADP+	92
Escherichia coli	Q15K-W37R and Q15R-W37R	30-fold higher Km for NADP+	92
Escherichia coli	gamma-R97Q	10-fold increased Km for NAD	93
Escherichia coli	G252A	Reverse transhydrogenation activity	94
Pseudomonas fluorescens	K295A and K295M	104–106-fold lower turnover	95
M. thermoautotrophicum	R11K and R136K	Decreased Km	96
	Alkyl-dihydroxyacetonephosphate synthase		
Hansenula polymorpha	ts6 and ts44 mutant	Peroxisomes absent	97
	Dihydroxyacetone phosphate acyltransferase		
Corynebacterium glutamicum	S187C	Reduced enzymatic activity	98
	Triose phosphate isomerase		
Kluyveromyces lactis	**Kltpi1 mutant**	Loss of enzymatic activity	52
Plasmodium falciparum	Y74G	Reduced stability	53, 54
Plasmodium falciparum	C13D	7-fold reduction in the enzymatic activity	53, 54
Trypanosoma brucei	W12F	Reduced stability	55
Leishmania mexicana	E65Q	Increased stability	56
K. lactis	**DeltaTPI1 mutants**	Complete loss of activity	57
Bacillus stearothermophilus	N12H	Prevent deamidation at high temperature	99
Vibrio marinus	A238S	catalytic activity reduced	58
Trypanosoma brucei	C14L	Reduced stability and altered kinetics	59
Saccharomyces cerevisiae	K12R	Vmax reduced by a factor of 180, Km elevated	60
Saccharomyces cerevisiae	**K12H**	No catalytic activity at neutral pH	60
Saccharomyces cerevisiae	E165D	1000-fold reduction in catalytic activity	61
Salmonella typhimurium	R179L	Reduction in binding affinity	62
Trypanosoma brucei	H47N	Reduced stability	63
Escherechia coli	E165D	1000-fold reduction in specific activity	64
Escherechia coli	N78D	Lowered Kcat	65
Saccharomyces cerevisiae	H95G	400-fold decrease in catalytic activity	66
	Non-enzymatic protein		
	DHAP transporter		
Saccharomyces cerevisiae			100
mycoplasma mycoides			101
E. coli			102
Pseudomonas aeruginosa			103
Escherichia coli			104
Escherichia coli			105
Escherichia coli			106
Escherichia coli	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		
Escherichia coli	E231K	0.24% wild-type kcat	108
Escherichia coli	H153Q	3.5-fold increase in Km	108
Escherichia coli	H209Q	7.6-fold increase in Km	108
Escherichia coli	H257Q	19-fold increase in Km	108
	xylulose kinase		
Escherichia coli	XylB- mutant	Lack of growth on xylitol	109
	Dihydroxyacetone synthase		
Hansenula polymorpha	Pex1–6(ts)	Peroxisome-deficient	110
Hansenula polymorpha	Deltapex14	Lack normal peroxisomes	111
	Non-enzymatic proteins		
	Xylulose-5-phosphate receptor		
Mycobacterium tuberculosis			112
	Xylulose-5-phosphate trasporter		
Arabidopsis sp.			113

Proteins that bind 3-phosphoglycerate/3-phosphoglyceraldehye

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, Quinolinate 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

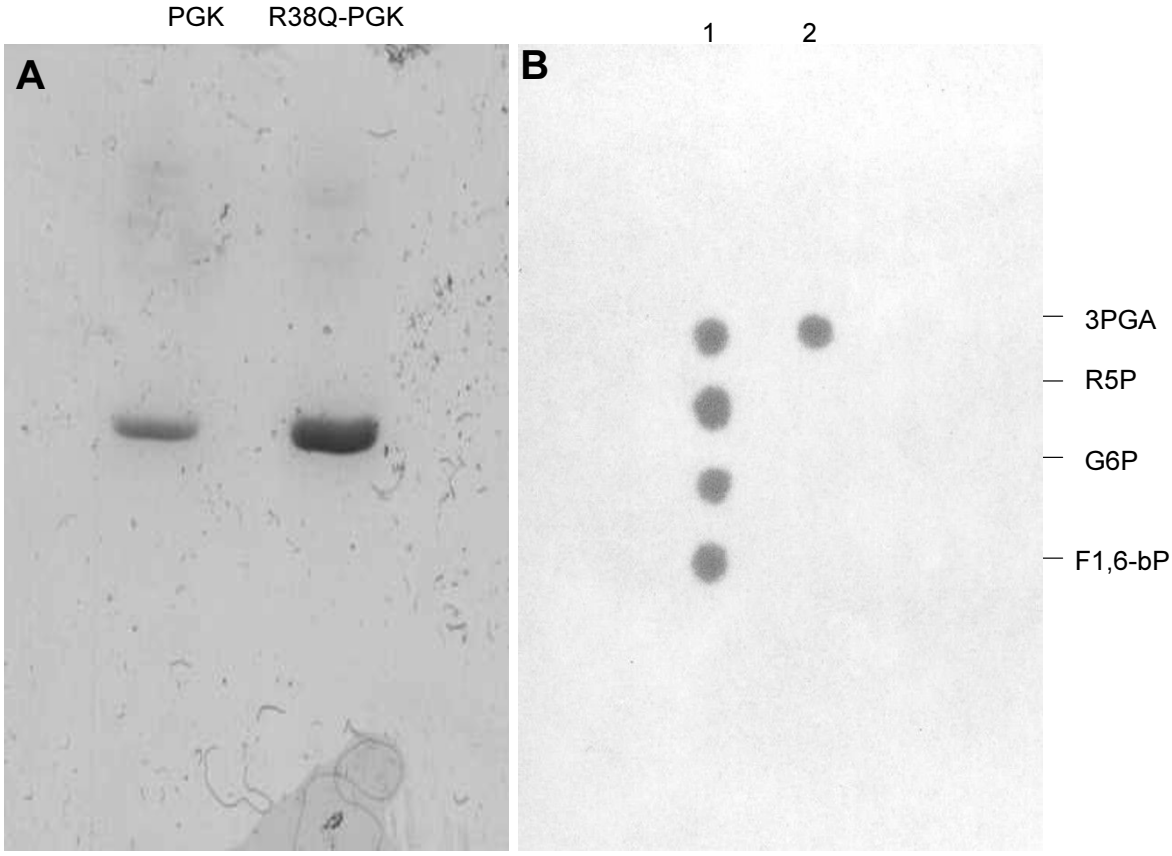

Figure 3
The recombinant his-tagged wild-type and R38Q mutant 3-phosphoglycerate kinase was subjected to affinity purification on Ni-NTA column as described previously [20]. **A.** SDS-PAGE of recombinant wild-type and R38Q mutant *S. cerevisiae* 3-phosphoglycerate kinase. The proteins (1 and 1.8 μg respectively) was separated in 10% polyacrylamide gel and stained with Coommassie 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-posphate (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	
Chamydomonas reinhardtii	C256F, K258R, L265V	85% decrease in Catalytic efficiency (V_{max}/Km)	114
Chamydomonas reinhardtii	G54V	83% decrease in the carboxylation-V_{max}	115
Anacystis nidulans	L339F, A340L, S341M	Decrease in Kcat and (V_{max}/Km) by 90%and 36.3% respectively	116
Anacystis nidulans	T342I, K343L	Decrease in Kcat and (V_{max}/Km) by 90%and 36.3% respectively	116
Anacystis nidulans	T342I	Decrease in Kcat and (V_{max}/Km) 40.5%and 40.5% respectively	116
Anacystis nidulans	K343L	Decrease in Kcat and (V_{max}/Km) 48.1%and 18.5% respectively	116
Anacystis nidulans	V346Y, D347H, L348T	Inactive	116
Anacystis nidulans	L326I	Decrease in Kcat and (V_{max}/Km) 54.4%and 34.2% respectively	116
Anacystis nidulans	S328A	Decrease in Kcat and (V_{max}/Km) 5.6%and 41.5% respectively	116
Anacystis nidulans	N123H	16.5% decrease in Kcat	116
Anacystis nidulans	L332M, L332I	>65% decrease in carboxylase but not in oxygenase activity	117
Anacystis nidulans		>65% decrease in carboxylase but not in oxygenase activity	117
Anacystis nidulans	L332V	67% decrease in specificity factor (CO_2/O_2)	117
Anacystis nidulans	L332T	67% decrease in specificity factor (CO_2/O_2)	117
Anacystis nidulans	L332A	>65% decrease in specificity and carboxylase activity	117
Rhodospirillum rubrum	deleation of F327	99.5% decrease in carboxylase activity	118
Rhodospirillum rubrum	F327L	Increase in Km (RuBP)	118
Rhodospirillum rubrum	F327V	Increase in Km (RuBP)	118
Rhodospirillum rubrum	F327A	Increase in Km (RuBP)	118
Rhodospirillum rubrum	F327G	165-fold increase in Km (RuBP)	118
Rhodospirillum rubrum	N111G	Km(RuBP), kcat are 320 fold increased and 88-fold decreased	119
Rhodospirillum rubrum	N111L	Mutant show a very low carboxylase activity	119
Rhodospirillum rubrum	N111Q	Mutant show a very low carboxylase activity	119
Rhodospirillum rubrum	N111B	Mutant show a very low carboxylase activity	119
*Synechococcus sp.*PCC6301	I87V	Mutant show a very low carboxylase activity (kcat = 35%)	120
*Synechococcus sp.*PCC6301	R88K	Mutant show a very low carboxylase activity (kcat = 35%)	120
*Synechococcus sp.*PCC6301	G91V	Mutant show a very low carboxylase activity (kcat = 35%)	120
*Synechococcus sp.*PCC6301	F92L	Mutant show a very low carboxylase activity (kcat = 35%)	120
*Synechococcus sp.*PCC6803	C172A	40–60% decline in Rubisco turnover number	121
Chlamydomonas reinhardtii	N123G	Decrease in specificity factor	122
Chlamydomonas reinhardtii	S379A	Decrease in specificity factor	122
Anacystis nidulans	**S376 C**	99% and ~99.9% decrease in carboxylase and oxygenase activity	123
Anacystis nidulans	**S376T**	99% and ~99.9% decrease in carboxylase and oxygenase activity	123
Anacystis nidulans	S376 A	99% and ~16% decrease in carboxylase and oxygenase activity	123
Rhodospirillum rubrum	I164T	6% decrease in carboxylase activity with 40-fold lower Kcat/Km	124
Rhodospirillum rubrum	I164N	1% decrease in carboxylase activity with 900-fold lower Kcat/Km	124
Rhodospirillum rubrum	I164B	0.01–1% decrease in carboxylase activity	124
Rhodospirillum rubrum	H287N	10^3-fold decrase in carboxylation catalysis	125
Rhodospirillum rubrum	H287Q	10^5-fold decrase in carboxylation catalysis	125
Rhodospirillum rubrum	M330L		126
		Rubisco (large subunit)	
Chamydomonas reinhardtii	R59A	Decrease in V_{max} for carboxylation reaction	127
Chamydomonas reinhardtii	Y67A	Decrease in V_{max} for carboxylation reaction	127
Chamydomonas reinhardtii	Y68A	Decrease in V_{max} for carboxylation reaction	127
Chamydomonas reinhardtii	D69A	Decrease in V_{max} for carboxylation reaction	127
Chamydomonas reinhardtii	R71A	decrease in V_{max} (for carboxylation reaction) and thermal stability	127
Chamydomonas reinhardtii	A222T, V262L, L290F	Improved specificity factor and thermal stability	128
		Phosphoribulokinase	
Rhodobacter sphaeroides	T18A	8-fold decrease in V_{max}	129
Rhodobacter sphaeroides	S14A	40-fold decrease in V_{max}	129
Rhodobacter sphaeroides	S19A	500-fold and >1500-fold decrease in V_{max} and V_{max}/Km of RuBP	129
Rhodobacter sphaeroides	K165M, K165C	10^3-fold decrease in catalytic activity	130
Rhodobacter sphaeroides	R168Q	>300-fold decrease in catalytic efficiency	131
Rhodobacter sphaeroides	R173Q	15-fold decrease in V_{max}, 100-fold increase in Km for RuBP	131

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

Chlamydomonas reinhardtii	**R64C**	Almost inactive	132
Chlamydomonas reinhardtii	R64A	Decrease in activity	132
Chlamydomonas reinhardtii	R64K	Decrease in activity	132
Synechocystis sp.	S222F	Retains one-tenth catalytic activity	133
Rhodobacter sphaeroides	H45N	40-fold increase in Km for RuBP	134
Rhodobacter sphaeroides	N49Q	200-fold increase in Km for RuBP	134
Rhodobacter sphaeroides	K53M	No effect on catalysis or substrate binding	134
Rhodobacter sphaeroides	D169A	V_{max} diminished by 4-orders of magnitude	135
Rhodobacter sphaeroides	D42A	V_{max} diminished by 5-orders of magnitude	135
Rhodobacter sphaeroides	D42N	V_{max} diminished by 5-orders of magnitude	135
Rhodobacter sphaeroides	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 stategene 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-posphate (F1,6-bP) each at a concentration of 10 mM in a volume of 200 μl. 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 CO_2 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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