Ref.: Ms. No. BREV-D-17-00135
Identification of UDP-glucose binding site in glycosyltransferase domain of sucrose phosphate synthase from sugarcane (Saccharum officinarum) by structure based site-directed mutagenesis
Biophysical Reviews

Dear Mr Sugiharto,
I am pleased to report that the Reviewers have commented positively on your Biophysical Reviews contribution. They have, nonetheless, made a few suggestions that they believe would make your review even better. Consequently, I would be delighted to receive an amended manuscript from you which addresses the minor points listed at the end of this email. Alternatively, the Reviewers' comments can be accessed by following the provided link.

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When revising your work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript.
Your revision is due by 16 Nov 2017.
To submit a revision, go to http://brev.edmgr.com/ and log in as an Author. You will see a menu item called 'Submissions Needing Revision'. You will find your submission record there.

Please make sure to submit your editable source files (i. e. Word, TeX).

Yours sincerely
Damien Hall, Ph.D.
Executive Editor
Biophysical Reviews

Reviewers' comments:
Reviewer 1

Dear Editor,
Thank you for asking me to review the manuscript titled, ' Identification of UDP-glucose binding site in glycosyltransferase domain of sucrose phosphate synthase
from sugarcane (Saccharum officinarum) by structure based site-directed mutagenesis ', submitted by Bambang Sugiharto and coworkers.
The manuscript focused on the author’s work related to the identification of a binding site in a particular enzyme using bioinformatics, mutagenesis and enzyme activity assay techniques.
Please find some comments and some spelling/grammatical corrections below. I think that the review can be accepted after minor revision.
Best regards,

Reviewer

Comments
(1) Figure 2b. is explained as the affinity purification of His-tagged protein, but has no labelling in the figure. Lane 11 is supposedly the purified SPS but there are seems to be two major bands. Please add a marker to the figure to indicate the purified SPS, and possibly explain the extra bands(s).
(2) The manuscript seems to be missing an acknowledgments section and funding section.

P1
Abstract
line 32: consist of functional -> consists of a functional
line 33 glycosyltransferase domain -> the glycosyltransferase domain
line 36: as sugar acceptor -> as a sugar acceptor
line 38: the mechanism of -> of the mechanism of
line 39: alignments several enzymes, which -> alignments with several enzymes that
line 42: to the less -> to less
line 45: of UDP-G binding -> of the UDP-G binding
line 47: new strategy for -> new strategies for

P2
Introduction
line 6: Sucrose is a carbon mobile synthesized -> Sucrose is synthesized
line 10: reported that sucrose biosynthesis in prokaryotic -> reported on sucrose biosynthesis in prokaryotes
line 15: with -> which
line 16: include glycosyltransferase domain that regulates catalytic function of SPS -> including a glycosyltransferase domain that regulates the catalytic function of SPS
line 18: to be shorter -> to be a shorter
line 20: to be truncated the N terminal 20 kDa region off. -> to have a truncated N terminal 20 kDa region.
line 28: only glycosyltransferase -> only a glycosyltransferase
line 35: substrate binding site for-> substrate binding sites for
line 36: donor at glycosyltransferase -> donor at the glycosyltransferase
line 36: the previous study SoSPS1 has been used for -> SoSPS1 has been used in the previous study for
line 44: is critical process -> is a critical process
line 50: regulating in -> regulation in
line 54: that involved -> that are involved

P3
line 16: share the close similarity of glycosyltransferase -> share a close similarity in glycosyltransferase
line 30: three critical residues within that consist of R496, -> three critical residues, R496,
line 33: The previously studies -> In previous studies
line 35: In the structure of -> The structure of
line 36: 4RBN), it has proven -> 4RBN) has proven

line 41: residue is essential -> residues are essential
line 41: substrate UDP-G notably -> substrate UDP-G, notably
line 42: showed -> shows
line 44: higher conserved -> more similar
line 44: whereas not in -> compared to
line 45: have -> has
line 50: is appeared to -> appears to
line 53: interact with pyrophosphate -> interactions with the pyrophosphate

P4
line 6: characteristic -> characteristics
line 26: mutation has been -> mutagenesis has been
line 27: SoSPS1 with comparing the -> SoSPS1 by comparing the
line 30: saturating substrate UDP-G and F6P at concentrations 20 mM -> substrate UDP-G and F6P at saturating concentrations (20 mM)
line 35: S495 is significantly -> S495 significantly
line 32: structure of SPS. -> SPS structure.
line 35: is significantly altered the activity of enzyme. -> significantly altered enzyme activity.
line 38: If the mutation at S495 was carried out to replace Ser with Ala that adopts
from sequence conservation in SuSy, -> If Ser at S495 is replaced with Ala, it adopts the sequence conserved in SuSy and
line 42: of intramolecular hydrogen bond. -> of the intramolecular hydrogen bond.
line 44: mentioned that mutagenic replacement at the position R496 -> mentioned mutagenic replacement at position R496
line 46: emphasizes the -> , emphasizing the
line 48: in the less active enzyme compared to the wild type enzyme -> less active compared to the wild type enzyme.
line 51: other hand, we conducted further mutational analysis these -> the other hand, we conducted further mutational analysis on these
line 53: replacing with each Lys and Glu, respectively. -> replacing each with Lys and Glu, respectively.
line 58: these mutants without retained -> these mutations without retaining

P5
line 9: than interaction of -> than the interaction of
line 12/13: and consider as capability of binding substrate UDP-G. Thus, the predicted interaction between residues in SoSPS1 and UDP-G notably with diphosphate moiety has experimentally proven.
line 41: the plant SPS is showing substrate specificity to UDP-G, whereas the HoSPS is showing a -> plant SPS show substrate specificity to UDP-G, whereas HoSPS shows a
line 47: The previously report of SPS structure from H. orenii has been notified that the residue L324 which is corresponding to V570 in SoSPS1 is-> The previously reported SPS structure from H. orenii has shown us that residue L324, corresponding to V570 in SoSPS1,
line 50 other residue which is corresponding -> the other residue corresponding
line 52: involved in nucleotide sugar -> involved with nucleotide sugar
line 59: is important residue -> is an important residue

P5
line 5: in previous report showed that Val residue has highly conserved with other plant
-> in previous reports showed that Val residue is highly conserved in other plant
line 9: recently reported -> recently been reported
line 12: were occurred at -> were made at
line 12: the results were not
succeeded and the substrate preference showed no change, which still prefer to ADP -> the experiment did not succeed and the substrate preference showed no change, still preferring ADP
line 18: Similar to sucrose synthase that plant SPS showed a strong preference for UDP-G as a substrate, opposed to bacterial SPS that predicted to bind ADP-G. -> Similar to sucrose synthase, \plant SPS showed a strong preference for UDP-G as a substrate, as opposed to bacterial SPS, which are predicted to bind ADP-G.
line 26: This works are more challenging since a crystal structure of plant SPS has not provided yet. -> This work is challenging since a crystal structure of plant SPS has not been obtained yet.
line 27: Identify the specific -> Identifying the specific
line 32: for catalytic of -> for catalysis by
line 33: to altering substrate -> to alter substrate
line 33: prospect for structural -> prospects for structural

P7
Legend
line 12: The highly conserved and three critical residues -> The three highly conserved and critical residues
line 13: The residues that predicted involved -> The residues that are predicted to be involved

Comments
(1) Figure 2b. is explained as the affinity purification of His-tagged protein, but has no labelling in the figure. Lane 11 is supposedly the purified SPS but there are seems to be two major bands. Please add a marker to the figure to indicate the purified SPS, and possibly explain the extra bands(s).
(2) The manuscript seems to be missing an acknowledgments section and funding section.

Reviewer #1: Please see attached.

Reviewer 2

Journal
Biophysical Reviews

Manuscript title
Identification of UDP-glucose binding site in glycosyltransferase domain of sucrose phosphate synthase from sugarcane (Saccharum officinarum) by structure based site-directed mutagenesis

Manuscript number
BREV-D-17-00135

Comments:
“Accepted with (minor) revisions.

1. Page 5 text at line number 12, “was similar”, in fact data at Fig 2 (page 12) does not show exactly similar. My suggestion is the authors quantify the intensity of the bands using ImageJ or other software, and added the results in the text to justify the claim “similar”.
2. Page 5 text at line number 22-23, “purified samples were further assayed”, and page 8 text at line 34-35 (legend to fig 2), “Activity of SPS mutants ... and vector pTrcHis as control”. Please verify whether the data at Fig 2c were taken using purified samples? If yes, how did you prepare “purified samples from vector only containing E coli”? Please add your detail description in the manuscript, because it was confusing.
3. Page 5 text at line number 56-58, “reveals unexpected influence of these mutants without retained the enzymatic activity (data not shown)”. Does it mean the mutants completely loss of their activities? I suggest the data are shown.

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There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.
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Editorial Manager Registration

Inbox ×

Editorial Office Biophysical Reviews <em@editorialmanager.com> Thu, 28 Sep 2017, 19:47

to me

Dear Mr Sugiharto,

Thank you for registering for the Editorial Manager online submission and peer review tracking system for Biophysical Reviews.

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Your username is: BSugiharto
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Best regards,

Editorial Office
Biophysical Reviews

BREV: BREV-D-17-00135 - Submission Confirmation for Identification of UDP-glucose binding site in glycosyltransferase domain of sucrose phosphate synthase from sugarcane (Saccharum officinarum) by structure based site-directed mutagenesis

Inbox ×

Editorial Office Biophysical Reviews <em@editorialmanager.com> Fri, 13 Oct 2017, 11:04

to me

Dear Mr Sugiharto,

Your submission entitled "Identification of UDP-glucose binding site in glycosyltransferase domain of sucrose phosphate synthase from sugarcane (Saccharum officinarum) by structure based site-directed mutagenesis" has been received by Biophysical Reviews

The submission id is: BREV-D-17-00135
Please refer to this number in any future correspondence.

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author. The URL is http://brev.edmgr.com/.
Alternatively, please call us at 001-630-468-7784 (outside the US)/(630)-468-7784 (within the US) anytime from Monday to Friday.

Your manuscript will be given a reference number once an Editor has been assigned.

Thank you for submitting your work to our journal.

Kind regards,