THE
EMBO
JOURNAL

Manuscript EMBO-2010-73780

Crystal structure of the Yeast Sac1: Implications for Its Phosphoinositide Phosphatase Function

Andrew Manford, Tian Xia, Ajay Kumar Saxena, Christopher Stefan, Fenghua Hu, Scott D. Emr, Yuxin Mao

Corresponding author: Yuxin Mao, Cornell University

Review timeline:

Submission date:	26 January 2010
Editorial Decision:	19 February 2010
Revision received:	04 March 2010
Accepted:	08 March 2010

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 19 February 2010

Thank you for submitting your manuscript for consideration by The EMBO Journal. It has now been seen by two referees whose comments to the authors are shown below. As you will see the referees are very positive and support publication here. I would therefore like to invite you to prepare a revised manuscript in which the points raised by referee 2 are addressed in an adequate manner. I should remind you that it is EMBO Journal policy to allow a single round of revision only and that, therefore, acceptance of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process initiative, please visit our website: http://www.nature.com/emboj/about/process.html

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,
Editor
The EMBO Journal

REFEREE REPORTS:

Referee #1 (Remarks to the Author):

This is a very interesting manuscript that reports the crystal structure of the yeast phosphatase Sac1. This is the first solved structure for the Sac protein family. It highlights a novel structural fold that is conserved in the Sac family members that suggests a novel catalytic mechanism for Sac1. It also provides interesting insight that may help in our understanding the cis-mode versus trans-mode of action of the phosphatase.

The data are clearly presented and discussed.

Referee #2 (Remarks to the Author):

This manuscript describes the crystal structure of the Sac domain of yeast Sac1, representing the first atomic structure of the Sac family phosphoinositide phosphatases. These enzymes play important roles in dephosphorylating phosphoinositides and mutations of Sac-encoding genes have been linked to neurological disorders. The structure reveals the Sac domain is composed of an N-terminal (SacN) domain of a completely novel architecture, and a catalytic domain that resembles (but differs in detail) from the PTP and DSP domains of Cys-based protein tyrosine phosphatases, dual specificity phosphatases and lipid phosphatases of the PTEN and MTMR families.

The structure reveals some unusual and interesting features. First, the novel architecture of the SacN domain and its interface with the catalytic domain create a prominent cleft dominated by a positive electrostatic potential. Second, the opposite surface of the protein has a pronounced negative electrostatic potential, typical of membrane-associated proteins. A third striking feature is the unusual conformation of the catalytic PTP-loop.

This is an interesting manuscript that is appropriate for publication in EMBO J. A few points need to be addressed:

1. Although Sac is C(x)5R phosphatase, the catalytic domain has a different topology from conventional PTPs and the lmPTPs. However, these differences are not clearly indicated in the text and figures. Superimpositions of the Sac catalytic domain onto PTP1B and lmPTPs shown in stereo would be helpful.

2. The conformation of the PTP loop appears in an open conformation, rather similar to the conformation of PTP1B and RPTPa D1 in the oxidized (cyclic sulphenamide state). Is there any evidence that Cys392 is oxidized? The authors should show electron density for Cys392 and the PTP loop.

Minor points:

1. R256 should be labelled in Fig. 4b,c.
2. There are numerous spelling errors in the text that need to be corrected.
3. Superimpositions in Supp Figs 2 and 3 should be shown in stereo.

1st Revision - authors' response 04 March 2010

RESPONSES TO THE REVIEWERS' COMMENTS:

Referee #1 (Remarks to the Author):

This is a very interesting manuscript that reports the crystal structure of the yeast phosphatase Sac1. This is the first solved structure for the Sac protein family. It highlights a novel structural fold

that is conserved in the Sac family members that suggests a novel catalytic mechanism for Sac1. It also provides interesting insight that may help in our understanding the cis-mode versus trans-mode of action of the phosphatase.
The data are clearly presented and discussed.

We thank the reviewer for his/her very positive remarks.

Referee #2 (Remarks to the Author):

The structure reveals some unusual and interesting features. First, the novel architecture of the SacN domain and its interface with the catalytic domain create a prominent cleft dominated by a positive electrostatic potential. Second, the opposite surface of the protein has a pronounced negative electrostatic potential, typical of membrane-associated proteins. A third striking feature is the unusual conformation of the catalytic PTP-loop.

We thank the reviewer for his/her very supportive comments.

This is an interesting manuscript that is appropriate for publication in EMBO J. A few points need to be addressed:
1. Although Sac is C(x)5R phosphatase, the catalytic domain has a different topology from conventional PTPs and the lmPTPs. However, these differences are not clearly indicated in the text and figures. Superimpositions of the Sac catalytic domain onto PTP1B and lmPTPs shown in stereo would be helpful.

We performed structure comparison of Sac1 with both PRL-1 and LMW PTPs and generated new supplementary figure (supp Fig 2) to demonstrate the difference between Sac1 and other PTPs.

2. The conformation of the PTP loop appears in an open conformation, rather similar to the conformation of PTP1B and RPTPa D1 in the oxidized (cyclic sulphenamide state). Is there any evidence that Cys392 is oxidized? The authors should show electron density for Cys392 and the PTP loop.

We thank the review to raise the concern about possible oxidization of the catalytic cysteine. In fact, the protein that we used for crystallization is in a solution containing 10 mM DTT. Furthermore, in the 2fo-fc map (supp fig. 4), we didn't see any extra electron density that supports oxidized products.
We also compared the structure of PTP1B oxidized in the cyclic sulfonamide state (PDB ID: 1OEM) with Sac1. We found that the conformations of the P-loops are also strikingly different between the two proteins (supp fig 5), suggesting that the unique conformation of the p-loop of Sac1 is likely not due to oxidation.

Minor points:

1. R256 should be labelled in Fig. 4b,c.
We added the label of R256 to Fig. 4b. However, we didn't do so in Fig 4c due to the fact that the surface area contributed by R256 is not visible in this view.

2. There are numerous spelling errors in the text that need to be corrected.

We truly sorry for that, and hopefully we corrected them all in the revision.

3. Superimpositions in Supp Figs 2 and 3 should be shown in stereo.
We regenerated the superimposition figures in stereo.

© European Molecular Biology Organization