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Crystal structure of a prokaryotic homologue of the mammalian oligopeptide-proton symporters, PepT1 and PepT2.

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(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 October 2010

Thank you for submitting your manuscript for consideration by the EMBO Journal. It has now been seen by three referees whose comments are enclosed. As you will see, referees 1 and 2 are overall positive about your study and support publication pending appropriate revision - largely to the text. Referee 3, on the other hand, raises serious concerns in terms of the resolution of the data, and consequently does not recommend publication. Given the rather contradictory nature of these reports, I decided to involve an additional editorial advisor, who has taken a look at the manuscript as well as at the conflicting reports. He/she agrees with referees 1 and 2 that, while low resolution, the structure is well supported by the data and does provide valuable insight into this transporter.

Consequently, given the support of 3 expert referees, we would like to invite you to submit a revised version of your manuscript, addressing the comments of referees 1 and 2 (and 3 where appropriate). Particularly important would be to modify your text to better emphasise that the density observed in the ligand binding cavity does not necessarily represent bound peptide - an issue raised by all three referees. I would also strongly encourage you to cite and discuss the relevant literature highlighted by referee 2.

I should add that it is EMBO Journal policy to allow only a single round of revision. Acceptance of your manuscript will thus 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

We generally allow three months as a standard revision time, and as a matter of policy, we do not consider any competing manuscripts published during this period as negatively impacting on the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

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

Yours sincerely,

Editor
The EMBO Journal

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REFEREE REPORTS:

Referee #1 (Remarks to the Author):

This paper describes the X-ray structure of PepTSo, a bacterial homolog of the human PepT1 and PepT2 proteins. This is a great advance in understanding proton-driven peptide transport across membranes, and there are various reasons in support of publication:

- This is the first structure of a major facilitator specific for peptides, which makes PepTSo a great model system to study the mechanism not only of bacterial, but also of human peptide transporters. These have a very important physiological function and are likely also drug targets. Hence, having a bacterial model system with a solved structure at hand will greatly aid in understanding the molecular transport mechanism.

- Although the structures of other MFS transporters have been reported, PepTSo has an additional two transmembrane helices, a feature previously unknown (to my knowledge). This demonstrates how much we still have to learn and that many more structures are to be determined before we understand how MFS transporters work. The present study is a milestone and will be widely read and cited.

- PepTSo was found to adopt an occluded conformation, with an unknown substrate (modeled as a dipeptide) in a central binding pocket. This is different from the previously reported EmrD structure, where a similar conformation was reported, but without a bound substrate, which had raised questions about the physiological relevance of the EmrD study. With PepTSo, there is no doubt that the authors have captured a relevant transport intermediate.

- Even though the resolution of the study is relatively low, the methods indicate that great care has been taken to make sure the register of the side chains is correct. I have high confidence in the work of the authors.

This is a splendid paper that definitely merits publication. The comments below may improve the readability or clarify uncertainties:

1. The resolution is modest, and I'm a little surprised at the very good refinement R-factors, but in particular the small split between Rfree and Rwork. Has good care been taken to choose test reflections such as to minimize the effects of the three-fold NCS symmetry?

2. The functional data suggest a Km in the millimolar range. It would be surprising if a simple dipeptide was bound so tightly in the central binding pocket and remained there during purification.

Perhaps the authors should emphasize in the text that the identity of the bound substrate is unknown and that the observed density cannot reflect one of the dipeptides used for functional studies (those would bind much weaker).

Referee #2 (Remarks to the Author):

I read this article several times. In each case I had few or no comments that would be of substantial impact or that would really improve on this nice article. I believe the authors have convincingly documented the "occluded" state of an MFS transporter, and that this will provide excellent grounds for reconstructing the transport cycle once an open-to-the-outside form is found. (This is rumored to in the works, if not already submitted from another lab.) The authors should be pleased with their contribution; I am.

There were two minor and niggling points that occurred to me, but I am happy if they are ignored. (1) There is plenty of bioinformatic evidence about the different roles one presumes for the N- and C-terminal six helix bundles. Citing and discussing this would make the authors' argument (now based largely on LacY) stronger. (2) the bound substrate is modeled as a dipeptide. How certain are the authors that this is not the head group of DDM? It is not uncommon for bound detergent to be present in such structures, as I am sure that authors are aware.

Referee #3 (Remarks to the Author):

The paper reports the structure of prokaryotic homologue of the mammalian oligopeptide-proton symporter. The protein structure belongs to the MFS superfamily, there are some significant differences (addition of two helices) but these are not thought to be functionally crucial. The specificity of the channel for the oligopeptides is shown to be similar to others, di and tri go though but tetra is too big. The structure is then used for a molecular dynamics analysis of the transport. The novelty in the paper is the description of the closed conformations at both ends and electron density in the middle. The electron density is assigned as the dipeptide. This is taken to locate a binding cavity. The data are reliable enough to position the helices. The molecular modelling and analysis shed some light on the channel's function.

There are a number of technical deficiencies that preclude publication however.
(1) The resolution and quality of the data.
The data are anisotropic, the stats refer to the untruncated data. I suspect the truncated data would show completeness of less than 60% for data beyond 4. The structure such as it is dominated by geometric restraints. The low clash score drives the molprobity , the 1 in 6 wrong rotamers tells a different story, the structure has serious and given the data probably uncorrectable errors.
(2) The structural data are being over interpreted.
The register is assigned definitively and without qualification. Worse side chain interactions are used, the side chain positions are at best guesses and 1 in 6 is wrong.
(3) The density for the peptide is unconvincing
With this data, it is a blob. There can be many other explanations truncation errors, solvent boundaries etc
(4) Fundamentally the novel insight is limited
The transporter transports similar peptides to others already known. The structure beyond two not essential helices is very similar to that known.

I expect the authors will respond robustly. However at the core of any paper has to be data. I know these projects are hard, getting this far is a technical achievement but the data is not just good enough to give the insight required.

1st Revision - authors' response 02 November 2010

Thank you very much for your kind invitation to submit a revised version of our manuscript entitled the "Crystal structure of a prokaryotic homologue of the mammalian oligopeptide-proton symporters, PepT1 and PepT2". We were delighted by the generally supportive comments of referees 1 and 2, and are appreciative of their suggestions, and your own, for ways in which we might improve the manuscript. In the light of these comments and suggestions, we have revised the manuscript as follows.

Referee 1 commented on the small split between the refinement R and Rfree values that we obtained. To minimize the effect of the non-crystallographic symmetry on the Rfree value, we were very careful to select reflections omitted from the refinement using the CCP4 program SFTOOLS, which allows the selection of free reflections from resolution shells as opposed to randomly throughout the data. In preparing the data for refinement we were also very careful to optimise the resolution limits through truncation of the structure factors. We observed a clear improvement in both the R and Rfree values following the use of truncated structure factors and proceeded through several rounds of optimisation before deciding on the resolution limits employed. We would like to stress that the model was very carefully built using O and that only standard model building and refinement methods were employed throughout this study. Early on in the refinement process it became clear that the refinement program BUSTER, combined with truncated structure factors and the use of TLS and strict NCS refinement options gave the best results, as detailed in the supplementary information section of the manuscript. We are fully confident in the accuracy of the model given the resolution of the data.

Both referee 1 and 2 raised the question of the identity and source of the unexplained density in the central peptide-binding pocket of the protein. We agree with the referees that this density is unlikely to represent either a di- or tri-peptide, based on the Km values we calculate for PepTSo and the low affinity for peptides exhibited by other members of this family. We instead believe it to be either a non-natural ligand or an inhibitor originating from the crystallisation or purification stages. We have now changed the text in the manuscript to more clearly state this probability. We do not, however, believe that the density is likely to represent a bound detergent or lipid molecule, because we donít observe any extension to the density, as would be expected in these cases. We hope these clarifications have now suitably addressed these comments of the referees.

Referee 2 commented that citing additional literature concerning the proposed role sharing between the N- and C-terminal domains of MFS transporters would strengthen our own conclusions discussed in the manuscript. This was an excellent suggestion and to this end we have now cited two additional publications. The first is a recent bioinformatics study, highlighting that residues involved in sugar and proton binding in LacY appear well conserved in distantly related MFS transporters from both pro- and eukaryotic kingdoms (Kasho et al, 2006). These residues are located in either the N- and C-terminal bundles respectively, providing further biochemical support for the general principle of role sharing between the two halves of MFS transporters that we propose from our structural analysis. The second is a review article discussing the structural basis of transport operating within secondary active transporters from diverse families. This recent review suggests the possibility that in LacY, the largest conformational changes may occur within the C-terminal domain of the protein, as we propose from our analysis (Boudker & Verdon, 2010).

Referee 3 raised concerns regarding the dominance of geometric restraints during the refinement and subsequent model building. We are of the strong opinion that the geometry of the present model is very reasonable, given the resolution of the data. We would argue that low R and Rfree values indicate a good fitting of the model to the density, and would be unobtainable if the refinement were dominated by geometric restraints alone. Again, we would like to stress that this model was built with careful attention to the data so as not to over interpret.

We hope that by making the changes to the manuscript listed, and by clarifying some aspects of the work, as detailed above, we have adequately addressed the concerns and comments raised by the referees. The revised manuscript accompanies this letter. Many thanks in advance for your consideration.

Additional References added to the manuscript.

Boudker O, Verdon G (2010) Structural perspectives on secondary active transporters. Trends Pharmacol Sci 31: 418-426

Kasho VN, Smirnova IN, Kaback HR (2006) Sequence alignment and homology threading reveals prokaryotic and eukaryotic proteins similar to lactose permease. J Mol Biol 358(4): 1060-1070

Acceptance letter 04 November 2010

Many thanks for submitting the revised version of your manuscript EMBOJ-2010-76046R. I have now had the chance to look carefully through it and your response to the referees comments, and I am satisfied that you have addressed the concerns raised in the previous round of review. I am therefore pleased to be able to tell you that we can accept the manuscript for publication without the need to go back to the referees.

You should receive the formal acceptance message shortly.

Thanks for choosing EMBOJ for publication of this study.
Besyt wishes

Editor

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