Peer Review Report

Review Report on Probing the enzymatic activity and maturation process of the EcAIII Ntn-amidohydrolase using local random mutagenesis

Brief Research Report, Acta Biochim. Pol.

Reviewer: Jacek LUBKOWSKI

EVALUATION

Q 1 ▸ Please summarize the main findings of the study.

The manuscript by Loch et al., presents results of probing the effects of random mutations in selected sections of EcAIII on physicochemical and enzymatic properties of this enzyme. Although generally this protein is referred to as β-aspartyl dipeptidase (by the E.C. classification and the SwissProt description), it is referred here as Nth asparaginase (member of class 2 asparaginases). It may be worth noting that the asparaginase activity, i.e. catalysis of the L-Asn → L-Asp reaction is at best a secondary function of this enzyme and the in vivo role of this reaction is unclear. According to authors, this report presents a sequel to earlier studies and describes catalytic and physicochemical properties of mutated variants, randomly selected within regions that are observed as variable in nature. Furthermore, assessed fragments were suspected as important for functions of this enzyme.

After reading this report it is somewhat disappointing that authors did not find any meaningful connection between the specific sequence (motif) of a assessed fragment and properties of the enzyme such as a propensity for the autocatalysis or the catalytic activity of mature, two chains enzyme. There are very few reports on asparaginase activity of class 2 asparaginases and for that reason this report certainly provides an interesting new information. I am supportive for accepting this manuscript for publication, however, before that, I suggest fixing several typos as well as some more substantial changes in the current manuscript.

Q 2 ▸ Please highlight the limitations and strengths.

see below

Q 3 ▸ Please comment on the methods, results and data interpretation. If there are any objective errors, or if the conclusions are not supported, you should detail your concerns

n/a

Check List

Q 4 ▸ Is the English language of sufficient quality?

Yes.

Q 5 ▸ Is the quality of the figures and tables satisfactory?

Yes.

Q 6 ▸ Does the reference list cover the relevant literature adequately and in an unbiased manner?

Yes.

Q 7 — Are the statistical methods valid and correctly applied? (e.g. sample size, choice of test)

Yes.

Q 8 — Are the methods sufficiently documented to allow replication studies?

No.

Q 9 — Are the results presented correctly and interpreted in light of previous knowledge?

Yes.

Q 10 — Do the discussion and conclusion address the research questions or hypothesis posed in the introduction?

Yes.

Q 11 — Are the data underlying the study available in either the article, supplement, or deposited in a repository? (Sequence/expression data, protein/molecule characterizations, annotations, and taxonomy data are required to be deposited in public repositories prior to publication.)

Yes.

Q 12 — Does the study adhere to ethical standards including ethics committee approval and consent procedure?

Yes.

Q 13 — Have standard biosecurity and institutional safety procedures been adhered to?

Yes.

Q 14 — Please provide your detailed review report to the editor and authors (including any comments on the Q4 Check List):

More significant problems

(1) In contrast to authors suggestions, I do not consider the mutant RDM11-11 as an interesting finding and a negative test in anti-leukemia cellular assays. Authors can buy for fraction of price (taking the lab effort) good-quality albumin, hemoglobin, etc. to play a role of the negative reference. I expect removing this "idea" from the manuscript. It is not interesting peculiarity.

(2) I suggest some caution in interpreting the Km and kcat values. In measuring the first one, it is recommended to measure the rate of reaction at the substrate (L-Asn) concentration exceeding the Km value by factor of at least 10. But that demands concentrations exceeding limit of the L-Asn solubility. Of course, we all are bound by "limits", however, to refer to data as kinetic parameters (kcat and Km) we also need quite an accurate substrate concentration for which an evaluation based on A280 is quite a rough estimate. When we add subjective evaluation of completeness of maturation than final numbers have really no physical meaning. I do not find justifiable calling these numbers "apparent" kinetic parameters. They are not "apparent" and should not be reported here.

Minor but important problems

Table S3 (there are several issues)

Overall: if mutagenesis did end-up with not mutation, then you should not complicate the table and a reader time. Instead, mention how many screened colonies happened expressing unmodified (wt) enzyme (and reduce size of the table by 1/3). That will also take care of ** sub-note. You don't need to emphasize your effort, as it displayed already in the (maybe modified) table S2.

In the table S3, I do not see any results for series RDM4 and RMD9. Does that mean that mutations in these regions didn't lead to any productive results?

In series RDM8, I can see references (most likely erroneous) to RMD7 - fix it.

In series RDM7, there is only one mutant. That seems rather a "shallow" screening of this region.

I expect the last two points to be either "fixed" or commented.

In the main text.

Introduction:

The 2nd line:

Instead of "that hydrolases ..." should be "that hydrolase".

The 8th line:

Instead of "with L-asparaginase activity ..." should be "with the L-asparaginase activity".

The third paragraph:

Instead of "As all other Nth-hydrolases ..." could be rather "As all known Nth-hydrolases ...".

QUALITY ASSESSMENT

Q 15	Originality	■■■■□
Q 16	Rigor	■■■□□
Q 17	Significance to the field	■■□□□
Q 18	Interest to a general audience	■■■□□
Q 19	Quality of the writing	■■■■□
Q 20	Overall quality of the study	■■■□□