Enzymatic Characterization of *Leishmania major* Phosphatidylethanolamine Methyltransferases *Lmj*PEM1 and *Lmj*PEM2

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Abstract

Phosphatidylcholine (PC) is the most abundant phospholipid in the membranes of the human parasite *Leishmania*. It is synthesized via two metabolic routes, the de novo pathway that starts with the uptake of choline, and the threefold methylation of phosphatidylethanolamine.

Choline was shown to be dispensable for *Leishmania*; thus, the methylation pathway likely represents the primary route for PC production. Here, we have identified and characterized two phosphatidylethanolamine methyltransferases, *Lmj*PEM1and *Lmj*PEM2. Both enzymes are expressed in promastigotes as well as in the vertebrate form amastigotes, suggesting that these methyltransferases are important for the development of the parasite throughout its life cycle.

Heterologous expression in yeast has demonstrated that *Lmj*PEM1 and *Lmj*PEM2 complement the choline auxotrophy phenotype of a yeast double null mutant lacking phosphatidylethanolamine methyltransferase activity. *Lmj*PEM1 catalyzes the first, and to a lesser extent, the second methylation reaction. In contrast, *Lmj*PEM2 has the capacity to add the second and third methyl group onto phosphatidylethanolamine to yield (lyso)PC; it can also add the first methyl group, albeit with very low efficiency.

Fig. 1. General PC biosynthetic pathways. 1. PEMT; 2. choline kinase; 3. phospho-choline cytidylyltransferase; 4. choline phosphotransferase; 5. PC synthase. DAG,diacylglycerol; DM, dimethyl; MM, monomethyl; P, phosphate.

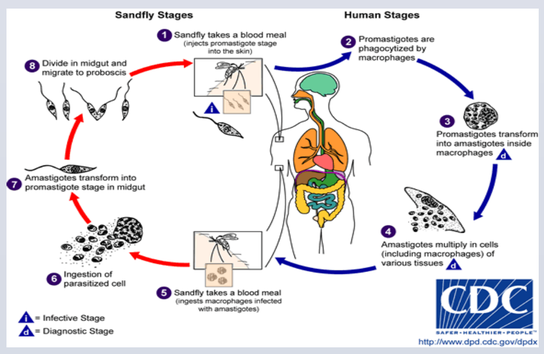


Fig. 2. Life cycle of *Leishmania*

Objectives

1. Establish that *Leishmania major* utilizes the methylation pathway involved in PC biosynthesis.
2. Identify putative *Leishmania major* genes involved in the PE methylation pathway.
3. Verify that these putative PE methyltransferase genes are expressed in *L. major* and determine their ability to complement auxotrophy in *Saccharomyces cerevisiae* lacking PE methyltransferase activity.
4. Determine substrate specificities of these enzymes

Results

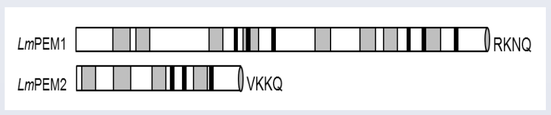


Fig. 3. Schematic representation of the PE methyltransferases LmjPEM1 and LmjPEM2 of *L. major*.
The grey rectangles represent the putative transmembrane spans, the black rectangles depict the conserved amino acids diagnostic of PE methyltransferases, and the grey ovals symbolize the putative endoplasmic reticulum retention motif.

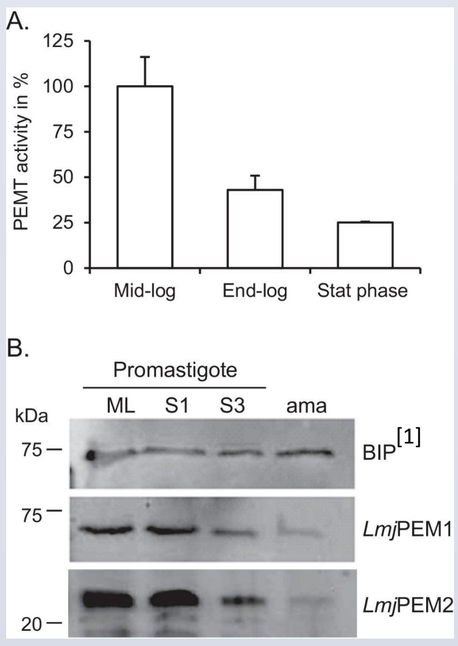


Fig. 3. *Lmj*PEM1 and *Lmj*PEM2 are expressed throughout the life cycle of *Leishmania*.

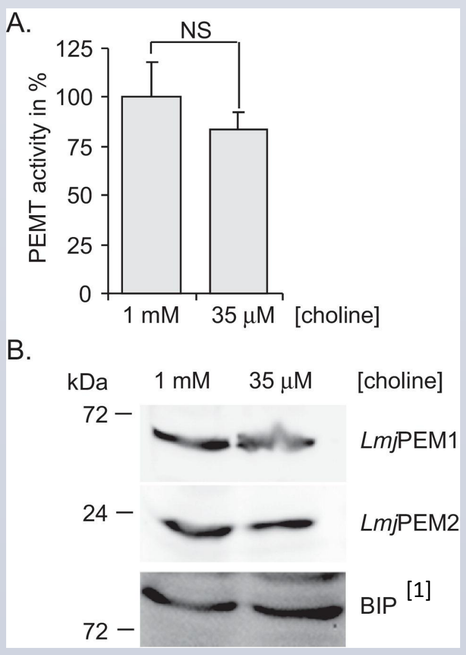
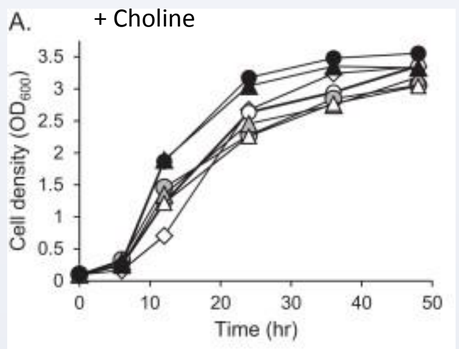


Fig. 4. Expression of *Lmj*PEM1 and *Lmj*PEM2 is choline independent.

Results



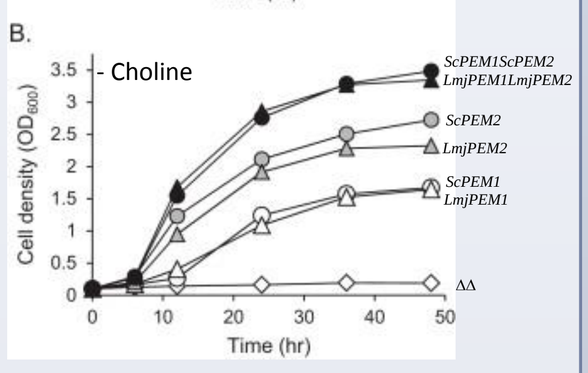


Fig. 7. *Lmj*PEM1 and *Lmj*PEM2 complement the choline auxotrophy phenotype of *S. cerevisiae* double null mutant *scpem1Δscpem2Δ* that lacks PEMT activity

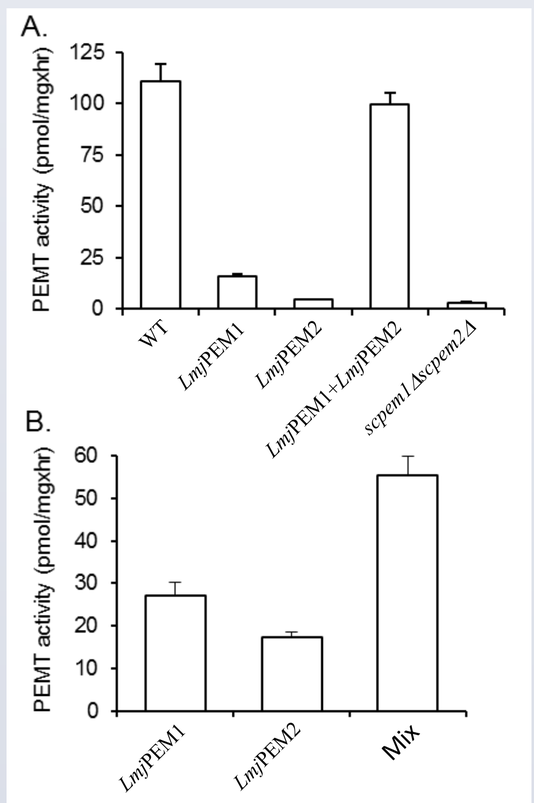


Fig. 8. LmjPEM1 and LmjPEM2 act as PEMT enzymes.

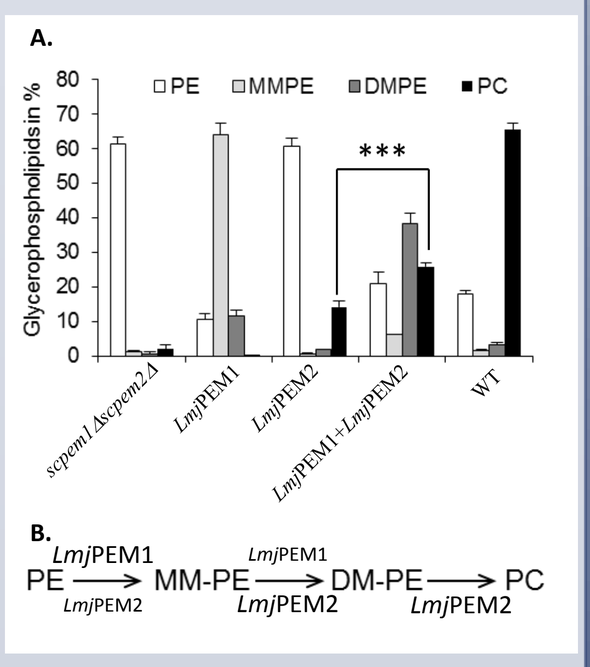


Fig. 9. *Lmj*PEM1 and *Lmj*PEM2 substrate specificities.

Conclusion

- ✓ *Leishmania major* posses two PE-methyltransferase genes, *LmjPEM1* and *LmjPEM2*
- ✓ *Leishmania major* express both *LmjPEM1* and *LmjPEM2* in both promastigotes and amastigotes in a cell cycle dependent manner
  - ✓ Which correlates to PE-methyltransferase activity
- ✓ Expression of *LmjPEM1* and *LmjPEM2* is independent of choline
  - ✓ As is PE-methyltransferase activity
- ✓ *LmjPEM1* and *LmjPEM2* complement the choline auxotrophy of *scpem1Δscpem2Δ* yeast deficient of PE-methyltransferase activity
- ✓ *Lmj*PEM1 catalyzes the first and second methylations of PE producing MM-PE and DM-PE
  - ✓ Albeit with lower affinity for the second methylation
- ✓ *Lmj*PEM2 catalyzes all three methylations of PE producing MM-PE, DM-PE, and PC
  - ✓ Albeit with a lower affinity for the first methylation

References

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Acknowledgements

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