Menaquinone-2 and ubiquinone-2 adopt folded, U-shaped conformations contradicting current dogma

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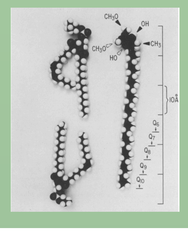
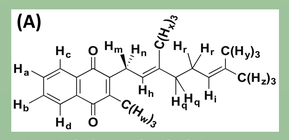
Background/Purpose

- Lipoquinones, such as ubiquinones (UBQ) typically found in eukaryotes and Gram-negative prokaryotes and menaquinones (MK) typically found in Gram-positive prokaryotes, including many pathogens such as *Mycobacterium tuberculosis*
- Lipoquinones are essential components of the electron transport chain and participate in cellular respiration, essential for life
- Little is known regarding the conformation of MK & UBQ
- UBQ is represented as a "Q" within the membrane in life science textbooks or in extended conformations in primary literature (Fig. 1)

Fig. 1 (right): Space filling models illustrating the extended dimensions of ubiquinone-10 relative to a phospholipid bilayer.[1]





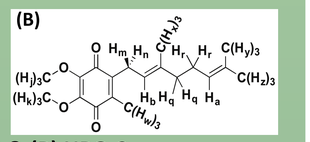
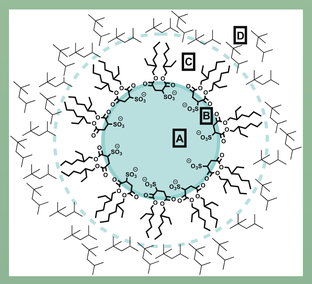


Fig. 2 (above): (A) Structure of MK-2 & (B) UBQ-2.

- The characterization of MK-2 & UBQ-2's (Fig. 2) conformation in organic solution and within the reverse micelle (RM) interface (Fig. 3) is important as these truncated analogs serve as reference compounds for naturally occurring UBQ-10 & MK-9
- It is likely that MK-9 & UBQ-10 also adopt folded conformations within the cellular membrane and thus impacting reactivity & function in important cellular redox mechanisms



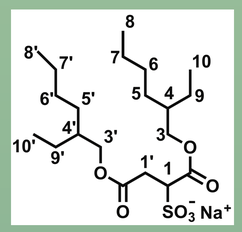


Fig. 3: RM present in a microemulsion (left). AOT proton labelling scheme key also shown (right).

Hypothesis

We hypothesize that MK-2 and UBQ-2 adopt folded conformations within organic solution and within the RM model membrane interface.

Experimental Design / Procedure

- Samples of either MK-2 or UBQ-2 were prepared and placed inside NMR tubes
- 2D NMR spectra were then collected using a NMR instrument & data analyzed to determine conformation of the molecule

Results/Outcomes

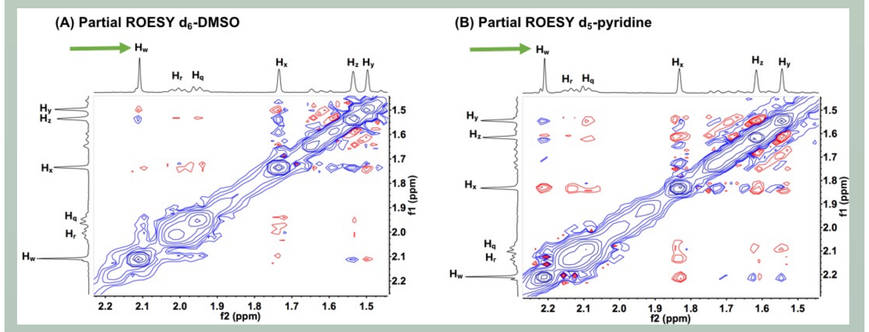


Fig. 4: ^{1}H-^{1}H 2D ROESY NMR (400 MHz) spectra of 20 mM MK-2 in d_6-DMSO and d_5-pyridine at 26 °C. (A) Full ^{1}H-^{1}H 2D ROESY NMR spectrum of MK-2 in d_6-DMSO, (B) Partial ^{1}H-^{1}H 2D ROESY NMR spectrum of MK-2 in d_5-pyridine.

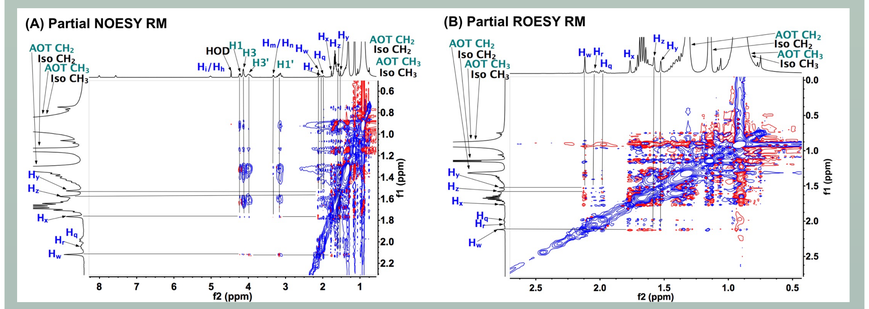


Fig. 5: Partial ^{1}H-^{1}H 2D NOESY NMR (400 MHz) spectra of MK-2 inside w_0 12 RM at 26 °C. (A) Full ^{1}H-^{1}H 2D NOESY NMR spectrum in w_0 12 RM and (B) Partial ^{1}H-^{1}H 2D ROESY NMR spectrum in w_0 12 RM.

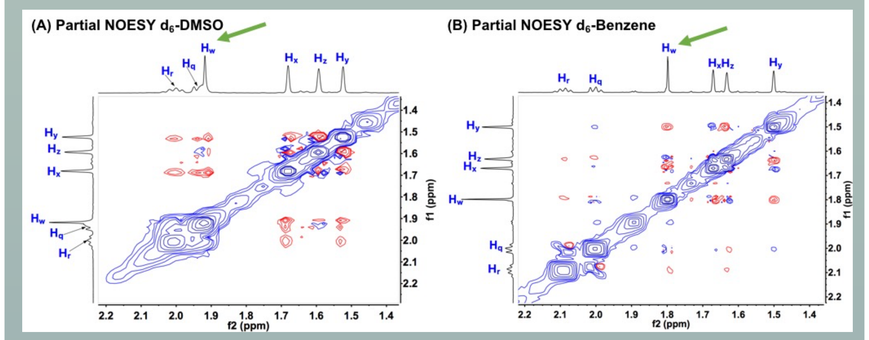


Fig. 6: ^{1}H-^{1}H 2D NOESY NMR (400 & 500 MHz) spectra of 20 mM UBQ-2 in d_6-DMSO and d_6-benzene at 26 °C. (A) Full ^{1}H-^{1}H 2D NOESY NMR spectrum of UBQ-2 in d_6-DMSO, (B) Partial ^{1}H-^{1}H 2D NOESY NMR spectrum of UBQ-2 in d_6-benzene.

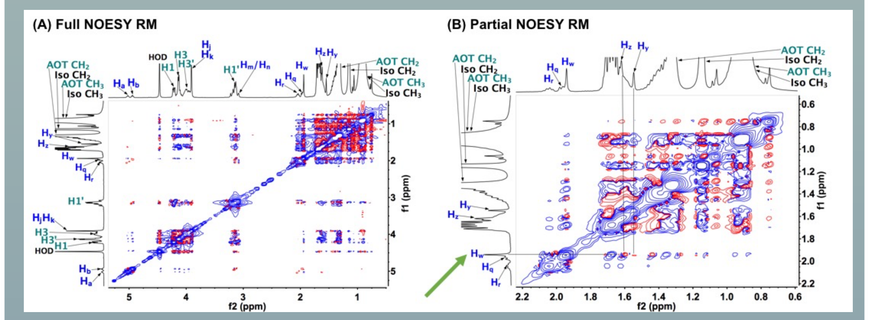


Fig. 7: Partial ^{1}H-^{1}H 2D NOESY NMR (400 MHz) spectra of UBQ-2 inside w_0 12 RM at 26 °C. (A) Full ^{1}H-^{1}H 2D NOESY NMR spectrum in w_0 12 RM and (B) Partial ^{1}H-^{1}H 2D NOESY NMR spectrum in w_0 12 RM.

Results/Outcomes Cont'd

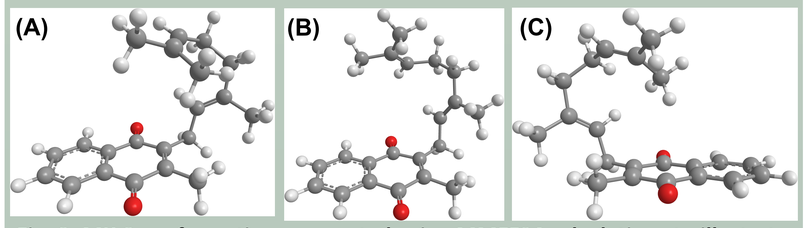


Fig. 8: MK-2 conformations generated using MMFF94 calculations to illustrate conformations elucidated by the 2D NMR spectroscopic studies. (A) Illustrates conformation in d_6-DMSO (H_w-H_y: 2.6 Å), (B) illustrates conformation in d_5-pyridine (H_w-H_y: 6.1 Å), and (C) illustrates conformation in RM (H_w-H_z: 4.0 Å).

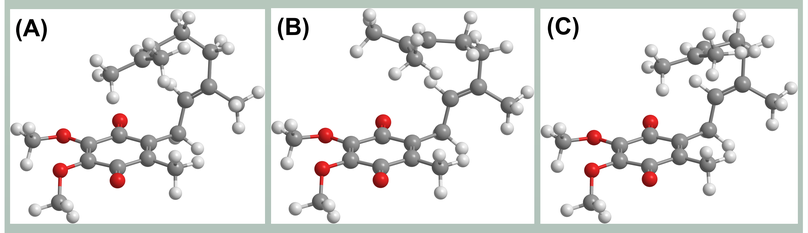


Fig. 9: UBQ-2 conformations generated using MMFF94 calculations to illustrate conformations elucidated by the 2D NMR spectroscopic studies. (A) Illustrates conformation in d_6-DMSO (H_w-H_y: 3.4 Å), (B) illustrates conformation in d_6-benzene (H_w-H_y: 3.6 Å), and (C) illustrates conformation in RM (H_w-H_y: 4.3 Å).

Conclusions

- MK-2 & UBQ-2 adopted similar folded, U-shaped conformations in organic solution
- MK-2 & UBQ-2 were located within the RM interface
- MK-2 & UBQ-2 adopted slightly different folded, U-shaped conformations within the RM model membrane interface
- Will change fundamental understanding & description of lipoquiones in literature

Impact/Future Directions

- Folded conformations of lipoquinones within the cellular membrane are likely to impact reactivity & function in important cellular redox mechanisms
- Analysis of native MK & UBQ analogs needs to be completed
- Analysis of partially saturated MK & UBQ analogs will provide insight if saturation affects conformation

References

1) Trumpower, B. L. "New Concepts on the Role of Ubiquinone in the Mitochondrial Respiratory Chain." *J. Bioenerg. Biomembr.* **1981**, *13*, 1-24.
2) Koehn, J.T., Magallanes, E.S., Peters, B.J., Beuning, C.N., Haase, A.A., Zhu, M.J., Rithner, C.D., Crick, D.C., Crans, D.C., "A synthetic isoprenoid lipoquinone, menaquinone-2, adopts a folded conformation in solution and at a model membrane interface." Submitted to *J. Org. Chem.*

Acknowledgements

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