DOUBLE ELECTRON-ELECTRON RESONANCE STUDIES OF THE CYTOPLASMIC DOMAIN OF BAND 3 PROTEIN

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Introduction

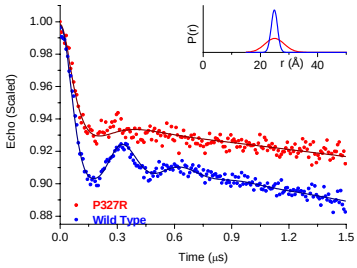
Double electron-electron resonance (DEER) spectroscopy has been used to elucidate the structure of the cytoplasmic domain of band 3 (CDB3) protein dimer at physiological pH and to determine what, if any, structural changes occur as a result of a Proline to Arginine mutation at residue 327. A crystal structure of the CDB3 dimer has been obtained at pH 4.8, however there is evidence of a pH-dependent large structural rearrangement [1]. The P327R mutation has been implicated in a heritable form of hemolytic anemia. The DEER capability of the pulsed X-band EPR spectrometer at the NHMFL makes it ideal for performing the long range distance measurements necessary to address these two issues.

Experimental

Single cysteine mutants of CDB3 have been expressed, purified, and spin-labeled with methanethiosulfonate spin label (MTSSL). DEER experiments were performed to measure the dipolar coupling between labels within the CDB3 dimer. A set of eight single cysteine mutants was designed to test for large domain rearrangements at physiological pH. A second set of seven single cysteine mutants (residues 339-345) along the dimerization helix of CDB3 was designed in both the wild type and P327R background to test for structural effects of the P327R mutation.

Results and Discussion

DEER measurements were successfully performed on all of the mutants described above. Preliminary analysis suggest that the distances obtained for the single cysteine mutants in the wild type background are consistent with the crystal structure obtained at low pH. A comparison of the results for the wild type and P327R backgrounds do not show evidence of structural rearrangement as a result of the P327R mutation. However, there is a significant increase in the width of the distance distribution at some sites.



DEER measurements of distance between MTSSL labeled L342C CDB3 in the wild type vs. P327R background.

Conclusions

The DEER measurements of CDB3 are consistent with the crystal structure obtained by Low and coworkers [1] at low pH. The P327R mutation causes an increase in the disorder along the dimerization helices.

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References

[1] Zhang, D., *et al*., *Blood*, **96**, 2925-2933 (2000).