STRUCTURAL INVESTIGATIONS OF APOLIPOPROTEIN-E FRAGMENTS BY NMR

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Introduction

Apolipoprotein-E (apoE) is responsible for cholesterol homeostasis and serves as a risk factor for diseases as diverse as Alzheimer’s and heart disease. The 22 kDa N-terminal domain (apoE1-181) undergoes a structural reorganization in the presence of the low-density lipoprotein (LDL) receptor[1]. Critical to the function of apoE is its LDL receptor-binding domain (apoE140-150). Intriguingly, the LDL receptor-binding domain has native-like activity when present as a concatemeric peptide (apoE140-150,140-150). 15N/2H enriched variants of apoE1-181 were used for structural investigations by 1H-15N TROSY HSQC. One-dimensional 1H-NMR experiments were accomplished with a concatemeric peptide from apoE’s receptor-binding domain (apoE140-150,140-150) to study the structural transition from inactive to active form.

Experimental

ApoE1-181: An 15N-1H TROSY HSQC experiment was recorded with 15N/2H labeled form apoE1-181 on the 600 MHz High Resolution 52 mm Superconducting NMR Magnet at 30 °C. Sample conditions were 10 mM d4-succinic acid (pH 5.5), 95:5 H2O:D2O. There were 4096 complex points collected in the direct dimension, 400 complex points in the indirect dimension, and 32 transients per increment. The carrier positions used in the acquisition were 15N, 120.0 ppm and 1H, 4.7 ppm. The spectral widths were 8000 Hz n the direct dimension and 2000 Hz in the indirect dimension.

ApoE141-150,141-150: 1-dimensional 1H spectra were acquired on the UnityInova 600 with a 2 mM sample of apoE141-150,141-150 in the presence and absence of 30% trifluoroethanol (TFE) at 4 °C. The spectral width of the experiments were 8000 Hz, the 1H carrier was set to 4.7 ppm, 4096 complex points were collected in 32 transients.

Results and Discussion

ApoE1-181: The results from the 15N-1H TROSY HSQC revealed far fewer peaks that the anticipated 181 15N-1H resonances. Upon examination of the sample at the end of the experiment, a white precipitant was observed and attributed to aggregation of the protein. The protein’s aggregation may result from being at the edge of the pI range (5<pI<7).

ApoE141-150,141-150: The 1-dimensional 1H spectra of the concatemeric peptide reveal little dispersion at 4 °C. The lack of dispersion suggests the peptide is mostly disordered in solution. Conversely, the dispersion in the amide region increases substantially in the presence of 30% trifluorothanol. Circular dichroism experiments suggested apoE141-150,141-150 is largely unstructured under these conditions; however, all twenty amide resonances are observable in the 1H NMR spectrum. The data argues for the presence of short-range order to the peptide.

Conclusions

Recently, reports in the literature have indicated that the presence of arginine and glutamate can be essential for the solubility and stabilization of difficult to prepare samples[2]. The next step of the proposed research is to prepare isotopically labeled variants of apoE1-181 for study by multidimensional NMR in the presence of stabilizing arginine and glutamate. For the apoE141-150,141-150concatemer, new sequences based upon the receptor-binding domain are being prepared to continue the study of the structure-dependent activity of these peptides.

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References