Structure Characterization of Apolipoprotein E C-terminal Domain

Chi-Jen Lo (駱啟仁)[1,2] and Ta-Hsien Lin (林達顯)[1,2,3]

[1]Institute of Biochemistry and Molecular Biology,
National Yang-Ming University, Taipei, Taiwan
[2]Structural Biology Program, National Yang-Ming University, Taipei, Taiwan
[3]Department of Medical Research & Education,
Taipei Veterans General Hospital, Taipei, Taiwan

Human apolipoprotein E (apoE) is a polymorphic protein of 299 amino acids with a molecular mass of ~34 kDa. It is composed of two independently folded domains (N-terminal and C-terminal domain) separated by a hinge region. The 22-kDa N-terminal domain (residues 1-191) and the 10-kDa C-terminal domain (residues 216-299) of apoE are responsible for receptor binding and lipid binding, respectively. The APOE genehas have three major alleles, ε2, ε3 and ε4, which encode three major isoforms, apoE2, apoE3, and apoE4, respectively. The three isoforms differ from one another only at residue 112 and 158, but have marked differences in their biological functions.ApoE has been known to play a key role in the transport of plasma cholesterol and lipoprotein metabolism. It is a major determinant in cardiovascular disease. ApoE is also highly associated with late-onset familial and sporadic Alzheimer's disease (AD). The lipid- and receptor-binding abilities of apoE are isoform-specific, suggesting that structural characteristics of apoE isoforms may play important roles in their biochemical functions. 3D structures of the N-terminal domain of apoE isoforms have been solved by x-ray crystallography, but the structural information of apoE C-terminal domain is limited.

The information of apoE(195-299) secondary structure was previously obtained from NMR experiments, which ApoE tends to form a tetramer and adopts a 90% of helical structure. Although we could obtain the information of secondary structure for ApoE, the detailed tertiary structure for ApoE/lipid is impeded by the molecular size and the complication of lipid environment. Alternatively, we hence applied SAXS to obtain the related structural information for ApoE, which SAXS can provide the radius of gyration for the protein particle and hence the protein/DHPC complex model could be built up.

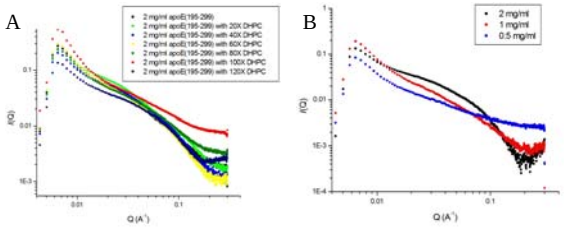

Fig. 1: (A) SAXS profiles of apoE(195-299) (2 mg/ml) in 50 mM phosphate buffer (pH 6.5) with different concentration ratio of DHPC, at 310 K. (B) SAXS profiles of apoE(195-299) (2/1/0.5 mg/ml) in 50 mM phosphate buffer (pH 6.5) with 100-fold DHPC, at 310 K.

The saturated protein:DHPC ratio was previously determined by NMR spectroscopy and was to be 1:100. In SAXS measurements, the ratio of ApoE/DHPC was varied and used to verify the distribution of protein-lipid particle size. As shown in Fig. 1 (A), the saturated protein:DHPC ratio was about 1:100, which is consistent with our NMR studies. At fixed protein/lipid molar ratio and by varying the protein concentration, the radius of gyration (Rg) for protein-DHPC complex showed to have two mode, which the Rg is obviously increased with the increase of protein concentration and reaches its saturation at protein concentration of 1 mg (Fig. 1B). Furthermore, as it can be seen in Fig 1(B), the molecular shape was also varied for protein concentration and showed to have two modes for protein concentration between 0.5 and 1 mg/mL. Using GNOM fitting program, the average Rg of the protein-DHPC particle was 57.2 ± 0.5 Å (Fig. 2). The dummy residues model was still in refinement.

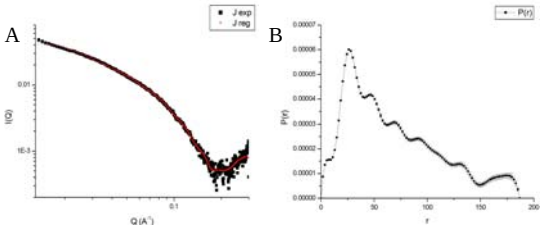

Fig. 2: (A) Scattering profile and normalization fitting with GNOM program for 2 mg/ml apoE(195-299) in 50 mM phosphate buffer (pH 6.5) with 100-fold DHPC, at 310 K. (B) Normalized results from GNOM program show the pairwise distance distribution P(r) and the maximum distance. The radius of gyration is fitted as 57.2 ± 0.5 Å.

Except for DHPC, DMPC and other lipid particle was also used for study, and a similar procedure was also applied. The related SAXS results are shown in Fig. 3. Unlike the results obtained in DHPC, the particle size for different protein concentration was to be the same. Only the molecular shape was different for the different concentration of lipid. So far, the process of data is still under going and will carry on the unfinished measurements in near future.

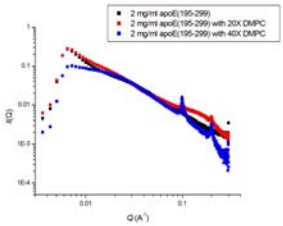

Fig. 3: SAXS profiles of apoE(195-299) (2 mg/ml) in 50 mM phosphate buffer (pH 6.5) with 20 and 40-fold DMPC, at 310 K.