The relation between dynamics and activity of phospholipase A/acyltransferase homologs

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Chapter

General discussion

The aim of this thesis was to study the reason for activity differences between the homologous enzymes PLAAT3 and PLAAT4. We hypothesized that differences in dynamics could play a role on the basis of observations accumulating over the years on the role of C-terminal (CTD) and N-terminal domains of these proteins. The transmembrane CTD was found to be crucial for PLAAT3 $PLA_{1/2}$ activity. Uyama *et al*.[1] showed that removal of this domain resulted in loss of phospholipase activity. In contrast, Golczak *et al.*[2] demonstrated that PLAAT4 truncated to its NTD has phospholipase activity, indicating that the transmembrane C-terminal domain is not critical. Golczak *et al.* also demonstrated that the rate of hydrolysis of short chain phosphatidylcholines of NTD of PLAAT4 is faster than the NTD of PLAAT3. Wei *et al.*[3] reported that C-terminal domains (CTDs) of both PLAAT4 and PLAAT3 can induce HeLa cell death at a comparable level, while their NTDs play opposite roles in regulating the cell death activity, even though their structures are highly similar. The NTD of PLAAT4 was found to be enhancing the cell death effect of the CTD, whereas the NTD of PLAAT3 was found to be inhibitory. These authors also complemented the observations of a previous study by Scharadin *et al.* [4] who found that residues 102-125, part of the loop renamed as L2(B6) in the thesis, of full-length PLAAT4 was necessary for pericentrosomal localization. Based on NMR studies, Wei *et al.*[3] hypothesized that the motif (residues 102-125) in PLAAT3 might be covered by the CTD while it was exposed in PLAAT4. Therefore, we hypothesized that there must lie a connection between the activities and overall dynamics contributed by amino acid differences between PLAAT3 and PLAAT4 NTDs and especially loop L2(B6).

The melting temperature of PLAAT3 is 15°C higher than that of PLAAT4 and the rotational correlation time is 33% shorter, showing that in solution PLAAT3 is a more stable and compact protein than PLAAT4. A comparison of the salt bridge networks show that the former has a more extensive network of salt bridges, which could explain these observations. Both proteins feature a large loop (L1) that is known to be disordered,[2,3,5,6] and is shown here to be mobile on the pico-nanosecond timescale. It has been proposed that L1 is a membrane anchoring loop in the full length protein.[6] It was shown that replacement of L1 in PLAAT3 by the equivalent loop of LRAT leads to induction of Vitamin A conversion.[6] This indicates that the highly disordered loop may play a role in modulating activity by interacting with the membrane. Interesting differences are found between PLAAT3 and PLAAT4 around the active site.

PLAAT4 shows low order parameters for R111 and N112, which immediately precede the catalytic nucleophile C113. Since there are no NMR resonance assignments for residues 106-110 and 113-114 in PLAAT4, it was assumed that the peaks of these residues are lacking because of line broadening due to motional chemical exchange. Millisecond timescale dynamics experiments identified a dynamic active site region involving residues 18, 19, 21, 23, 35 and 61. The resonance of G20 is missing, again indicative of exchange broadening. The peak of residue E22, which forms a salt bridge with R111, shows no broadening due to chemical exchange, which could imply that it either is not dynamic or its change in chemical shift ($\Delta\omega$) happens to be small. Thus, the active site region of PLAAT4 is dynamic, involving at least residues 18-23, 35, 61 and also 113-114. Such a dynamic patch is not observed in PLAAT3. We speculated that the difference in dynamics can explain the activity differences observed PLAAT3 and PLAAT4. The NTD of PLAAT3 may show little activity toward its substrate due to lack of active site dynamics, whereas the dynamics of PLAAT4 may enable its activity. To develop a model of what the dynamics in PLAAT4 may entail, molecular dynamics simulations were used. Although they can only sample fast dynamics, they still provided insight into the nature of motions and such calculations are known to correlate with NMR relaxation studies.[7–10] The RMSD profiles obtained from the MD simulations indicated that PLAAT4 is inherently more dynamic than PLAAT3, supporting the NMR results. Using PCA, it was also demonstrated that the loop L2(B6) in PLAAT4 shows concerted motions that are absent in PLAAT3.

Thus, these observations raised the question whether the mobility of L2(B6) relates to the activity difference between PLAAT3 and PLAAT4. Increased flexibility could play a role in substrate accessibility or induced fit. Introduction of the PLAAT4 L2(B6) loop indeed increased the activity of PLAAT3 strongly, though the opposite change, introducing L2(B6) from PLAAT3 into PLAAT4, did not reduce activity. By studying the lifetimes of the salt-bridges during MD simulations of L2(B6)-swapped structures, it could be observed that the introduction of a non-native L2(B6) disrupts the inherent network of salt bridges, the disruption being more prominent in PLAAT3, reducing some of the structural rigidity which might enhance activity of PLAAT_L2(B6)$_4$, perhaps, by increasing substrate accessibility or greater active site flexibility.

Apart from L2(B6), studying the interaction between L2(B6) and the highly flexible loop L1 in modulating catalytic activity (especially for PLAAT3) is another facet to a greater

understanding of the workings of these enzymes, because the two loops are adjacent to each other in NTD structures. However, in light of the models proposed by Golczak *et al.*[2] and Pang *et al.*[11] for full-length enzymes, the two loops are expected to be far apart when interacting with the membrane. Wei *et al.*[3] show that deletion of L1 in PLAAT3 enhances cell death inducing ability, not necessarily the phospholipase activity, since cell death inducing ability is conferred by the C-terminal domain. The authors mention that it is still not clear whether phospholipase activity plays any role in cell death. Therefore, the role of loop L1 in modulating phospholipase activity of PLAAT3 is unsure. We tried to address the question of interaction between loops L1 and L2(B6) in the NTD. Studying the interaction using NMR spectroscopy poses challenges since the NH-resonances of the residues constituting L1 are broadened beyond detection. The MD simulations show no evidence for contacts. The C_α distances between two pairs of residues in L2(B6) and L1 [Q102 and L105/K105 (PLAAT3/PLAAT4) in L2(B6) and A45 and V50 in L1] were calculated over the MD trajectory (Figure 6.1). The two loops, however flexible, do not interact significantly with each other.

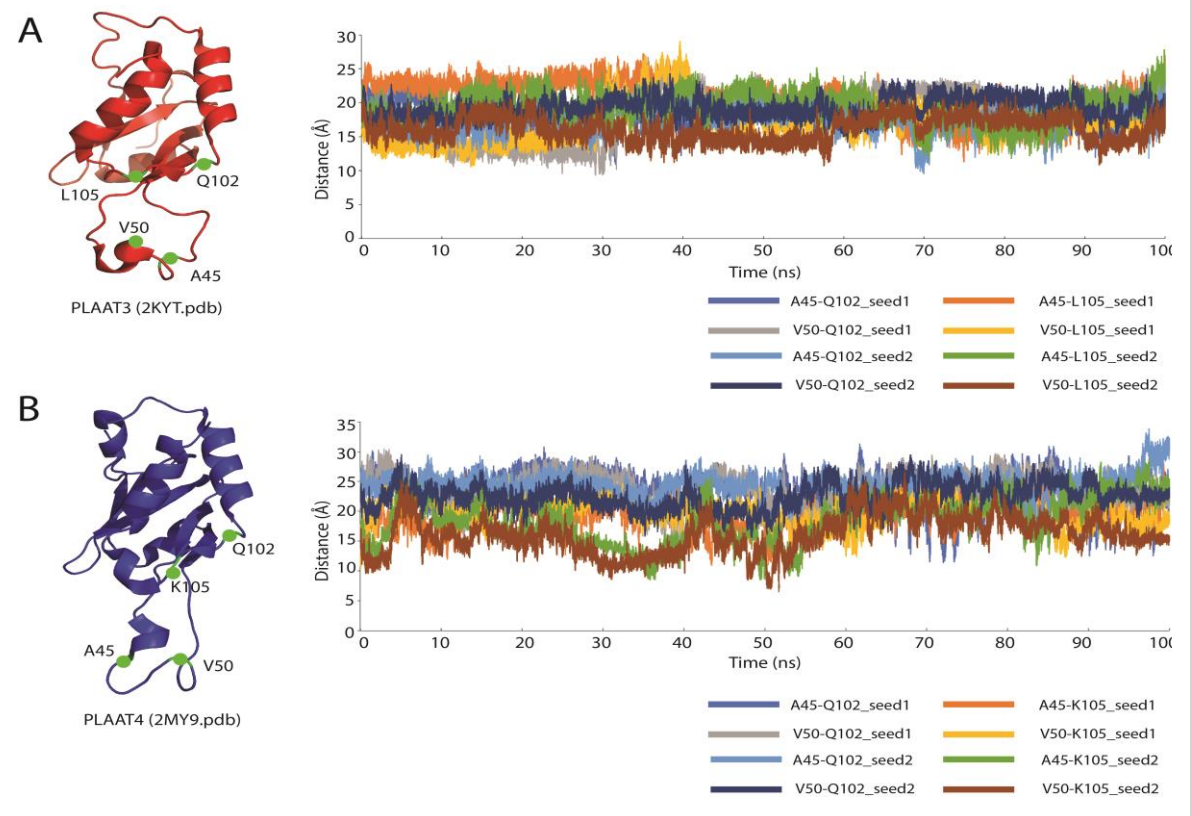

Figure 6.1 Distance plots between CA atoms of residues of L2(B6)-L105 (PLAAT3)/K105 (PLAAT4), Q102 and CA atoms of residues of L1- A45 and V50 in A) PLAAT3 and B) PLAAT4 during the course of MD trajectories (both seeds). L1 and L2(B6) were not seen to interact during the course of the trajectory and the residues of L1 maintain a distance of more than 5 Å or more with that of L2(B6).

Based on our findings, it can be concluded that PLAAT3 and PLAAT4 are dynamic proteins and the motions involve large parts of the structure. These motions clearly play an important role in modulating the activity, making it hard to explain activity differences with a simple model. For complete structural understanding of the activity difference of PLAAT family members, it will be important to compare the dynamics of the NTDs with those of the full-length proteins in a semi-native environment, such as a Nanodisc. Studying full-length proteins in Nanodiscs would also help decipher the role of the CTD and any possible interaction between the CTD and L1 loop, since L1 also interacts with the membrane and therefore should assume more define conformation than the highly flexible loop that is observed for the NTD. It would be interesting to see if the interaction of the CTD and L1 with the membrane bring about any conformational change or change in dynamics in the NTD of PLAAT3, thereby rendering it more catalytically active than NTD itself. This could shed more light on the role of PLAAT3 phospholipase activity in obesity[12], lens degradation[13] and in viral entry pathways.[14–16]

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