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主　論　文　の　要　旨

NMR analyses on recognition of the mitochondrial targeting signal by Tim50

論文題目　(Tim50 によるミトコンドリア移行シグナル認識機構の NMR 解析)

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論　文　内　容　の　要　旨

The immense majority of mitochondrial proteins are encoded by nuclear genes and are synthesized as precursor proteins on cytosolic ribosomes. Many precursor proteins use a targeting signal in the amino-terminal cleavable presequence for their targeting to mitochondria and subsequent sorting to the inner membrane or matrix via the TOM40 complex in the outer membrane and the TIM23 complex in the inner membrane. Tim50, a subunit of the TIM23 complex, plays multiple roles in protein translocation mediated by the TIM23 complex. As a presequence receptor, yeast Tim50 has a presequence binding site in not only the conserved core domain (residues 164-361) but also in the C-terminal “presequence binding” domain (PBD: residues 395-476). However, how presequences are recognized by the two distinct domains of Tim50 and how this presequence recognition is coordinated with the later step of translocation are not clear.

By testing several Tim50 PBD constructs with different lengths, residues 400-450 (sPBD) were found to be the most suitable for NMR analyses. NMR spectra of ^{15}N-labeled model presequence peptide, pSu9N (the N-terminal half of the presequence of subunit 9 of Fo-ATPase), were recorded with and without Tim50 sPBD or dTom20, the cytosolic receptor domain of yeast Tom20 in the TOM40 complex. The patterns of chemical shift changes are very similar between Tim50 sPBD and dTom20, suggesting that presequences are recognized in a similar manner at the outer membrane by Tom20 and at the inner membrane by Tim50 PBD. The affinity of Tim50 sPBD for pSu9N was

analyzed by titration of ^{15}N-pSu9N with increasing concentrations of sPBD, and found to be K_d of 0.1-0.3 mM, which is one order weaker than that of Tom20 for presequence peptides. To characterize the presequence binding site in Tim50 sPBD, NMR spectra of ^{15}N-labeled Tim50 sPBD were recorded with and without pSu9N or another presequence peptide. The presence of presequence peptides caused chemical shift changes for the signals arising from the two distinct segments, residues 410-415 and residues 433-439, in sPBD. This result indicates that two distinct segments at N- and C-terminal part of Tim50 sPBD are, probably collectively, responsible for interactions with presequences. Then, one or two mutation(s) was introduced into various positions in the two presequence binding segments in sPBD. Based on chemical shift changes of backbone amides of ^{15}N-labeled pSu9N upon addition of sPBD mutants, it can be concluded that both segments are involved in presequence binding. To indentify the nature of the binding forces between Tim50 sPBD and presequences, NMR spectra of ^{15}N-labeled Tim50 sPBD were recorded in the presence and absence of pSu9N(Q), where five hydrophobic residues around the sPBD binding element of pSu9N were replaced with hydrophilic glutamine. Binding of pSu9N(Q) to Tim50 sPBD was significantly suppressed as compared with pSu9N, suggesting that hydrophobic residues in pSu9N are important for its binding to sPBD. Supporting this interpretation, presequence-induced chemical shift changes around the C-terminal segment of Tim50 sPBD were enhanced at high salt concentrations, which is typical for recognition by hydrophobic interactions.

Next conserved Tim50core was added to the solution of ^{15}N-labeled Tim50 sPBD in NMR measurements. Many signals arising from the Tim50 sPBD residues that were found to be involved in presequence binding changed their chemical shifts and signal intensities as well. This indicates that Tim50core can bind to the presequence binding site in Tim50 sPBD. Interestingly, when Tim50core was added to the solution of ^{15}N-labeled Tim50 sPBD in the presence of pSu9N, the pattern of chemical shift changes upon Tim50core binding was different from that in the absence of pSu9N. This suggests that binding of Tim50core to Tim50 sPBD does not force the pre-bound pSu9N to leave from Tim50 sPBD completely, but instead lead to partial formation of the ternary complex consisting of Tim50core, Tim50 sPBD and pSu9N. Based on these results, a model for presequence recognition by Tim50 PBD and Tim50core was proposed and discussed.