

Functional analysis of *Thermus thermophilus* RecO

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RecF pathway is one of the eubacterial recombinational DNA repair pathway and responsible for DNA gap repair. Among many proteins involved in RecF pathway, RecF, RecO and RecR play the central role. Our previous studies suggest that RecO binds ssDNA, SSB and RecR and that RecO binds ssDNA tighter than SSB. We have been trying to determine the tertiary structure of *Thermus thermophilus* (tt) RecO and to analysis the interactions between RecO and other molecules, RecF pathway proteins or ss/dsDNA, by using NMR spectroscopy and biochemical approaches. In this presentation, we report the effect of ttRecO on ssDNA-dependent ATPase activity of ttRecA in the absence or presence of ttSSB, and the effect of ttRecR on that of ttRecO. In addition, results of NMR titration experiments of ¹⁵N-labelled RecO with RecR or other interacting proteins/DNA are discussed. ttSSB inhibits ssDNA-dependent ATPase activity of ttRecA. When ttRecO was added to the reaction, the inhibition was not recovered but was recovered in the presence of both ttRecO and ttRecR. The ATPase activity was maximally recovered when ttRecO and ttRecR exists about 1:2 molecular ratio. In the absence of ttSSB, ttRecO also inhibited ssDNA-dependent ATPase activity of ttRecA. However, the extent of the inhibition was smaller than that caused by ttSSB whereas ttRecO binds ssDNA much tighter than ttSSB. The inhibition by ttRecO was also recovered by adding ttRecR to the reaction. The most efficient recovery was observed when ttRecO and ttRecR exists about 1:2 molecular ratio. These results suggest that the inhibitory effect of ttSSB and ttRecO is regulated by the binding of ttRecR to ttRecO. ttRecR may modulate DNA binding affinity of ttRecO to release SSB bound-ttRecO or ttRecO from ssDNA easily. Finally, we demonstrated by the gel retardation assay that dissociation of ttSSB from ssDNA occur only when ttRecO, ttRecR and ttRecA all exist simultaneously. Therefore, we concluded that the dissociation of SSB results form extension of RecA filament.