E315 Dimerization of terminal protein domain of HBV polymerase

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HBV polymerase is important for their genome replication. Especially, terminal protein (TP) plays a essential role in protein-priming reaction. To reveal the biochemical characteristics of the protein, the terminal protein domain was expressed in *E. coli* and purified by Ni-NTA column. TP isolated by affinity column was further purified using gel filtration and anion exchange chromatography. Gel filtration chromatography and glycerol gradient sedimentation showed TP exists as dimeric form. In addition, we performed an experiment to determine the complex formation between MBP-fused TP(MBP/TP) and Histag-fused TP(His6/TP) by means of amylose column affinity chromatography. When the mixture of MBP/TP and His6/TP was applied to amylose resin, both MBP/TP and His6/TP were bound to the resin. It was detected by coomassie blue staining and western blot analysis. This experiment using the amylose resin revealed that MBP/TP and His6/TP form protein complex.

E316 Expression of Human Hepatits B Virus Polymerase by Baculovirus Expression System

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Human hepatits B virus(HBV) particle composed of envelope, core, polymerase and host cellular factor. Among those, polymerase is most important in HBV life cycle because polymerase replicate their genome. Polymerase is composed of terminal protein(TP), spacer, pol and RNase H(RH) domain. HBV polymerase - subtype *adr* - and each domains were expressed by baculovirus expression system to study HBV polymerase. Polymerase is tagged by flag epitope and domains of polymerase, those are RH domain and TP domain, are tagged by 6-histidine. Polymerase was purified by M2 immunoaffinity column. Purified polymerase was analyzed by SDS-PAGE and Silver staining and a prominent polymerase band with a molecular weight of approximately 90kDa was detected. Purified polymerase was examined by priming assays, labeled band appeared at the position of the Silver stained polymerase band. And also TP and RH were purified by Ni-NTA affinity column. In case of TP, insoluble fraction was purified high amount, but soluble fraction was not. RH was purified very low amount both fractions. Purified RH protein showed activity.