E317 Expression and Purification of RNase H domain of Human Hepatitis B virus(HBV) Polymeras

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The replication of HBV genome proceeds through a pregenomic RNA(pg RNA) intermediate. This pg RNA serves as the template for the formation of the viral DNA by the reverse transcriptase activity of the viral P gene product. The P gene product is believed to be a multifunctional enzyme with protein-priming, DNA polymerase, and RNase H activities. In HBV replication, the RNase H activity is essential in degradation of pg RNA as well as in generating the short RNA primer that is required for DNA second strand synthesis. However, detailed studies of this protein have not been performed because it could not be expressed in heterologous expression system. We used pBAD vector to overexpress the RNase H domain of the P gene product, fused with 6-histidine tag. As a result, we observed the protein in Coomassie blue staining and Western blotting. But the protein fails to fold into the native form when expressed in *E.coli*. Misfolded RNase H domain of HBV polymerase was dissolved in 6M Urea in 50mM Tris · Cl, pH 8.0. And the protein was purified using MODEL 491 Prep cell(Bio-Rad) by size fractionation. The pruified proteins showed a single band in silver-stained SDS-PAGE gel. The molecular weight of the proteins is consistent with that which was predicted form its amino acid composition.

E318 Overproduction and Functional Analysis of Recombinant Human Tumor Necrosis Factor-α Proteins

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We have tried to develop recombinant human TNFα proteins having increased antitumor activity and to establish an efficient way for the large-scale production of recombinant TNFα. Wild-type and several mutant TNFα cDNAs generated by PCR were subcloned into a bacterial histidine-tagged recombinant protein expression vector, pET28. The recombinant TNFα proteins overproduced in *E. coli* strain BL21 were purified to homogeneity from bacterial lysates by a single step using nickel affinity chromatography. TNFα proteins tagged with histidines at the N-terminus exhibited a similar cytotoxic activity towords WEH1 164 fibrosarcoma and L929 fibroblast cell lines to wild-type TNFα. However, TNFα proteins tagged with histidines at the C-terminus showed much lower cytotoxic activity than the wild-type. It thus appears that the introduction of the histidine tag at the N-terminus of TNFα ensures a rapid and efficient purification of the recombinant TNFα proteins retaining biological activities. Moreover, the cytotoxic activity of a mutant TNFα of which N-terminal 10 amino acids were substituted with three amino acids (arg-lys-arg) was much stronger than that of the wild-type. These results suggest that some modifications at the N-terminus of TNFα protein could increase its antitumor activity.