Evidence that the RNAseH activity of the duck hepatitis B virus is unable to act on exogenous substrates

ABSTRACT

These results imply that the RNAseH activity of the DHBV reverse transcriptase may not be able to degrade exogenous RNA:DNA heteroduplexes, although it can degrade heteroduplexes of the same sequence generated during reverse transcription of the endogenous RNA template. Therefore, the RNAseH activity appears to be "substrate committed" in a manner similar to the template commitment observed for the DNA polymerase activity.

INTRODUCTION

Background Hepadnaviruses are small, enveloped hepatotropic viruses which replicate by reverse transcription of a pregenomic RNA molecule [; for reviews see]. The replication of hepadnaviral DNA is initiated by the interaction of the viral reverse transcriptase (P) with an RNA stem-loop (∈) within a terminal duplication of the pregenomic RNA. P binds to the 5' copy of ∈ and then begins DNA synthesis, using a tyrosine residue near its own amino-terminus as a primer. DNA synthesis arrests after 3–4 nucleotides, and then the protein-linked nascent minus-strand DNA is transferred to the copy of direct repeat 1 (DR1) within the 3' terminal duplication. This nascent DNA then acts as a primer for the synthesis of full length minus-strand DNA templated by the pregenomic RNA. During synthesis of the minus-strand DNA the pregenomic RNA is degraded by the ribonuclease H (RNAseH) activity of P. However, the RNAseH activity does not digest the extreme 5' end of the template RNA, and this ~ 18 nucleotide capped RNA fragment is then transferred to direct repeat 2 (DR2), where it acts as a primer for synthesis of plus-strand DNA. The hepadnaviral P protein possesses many unique features relative to the better studied reverse transcriptases of the retroviruses: (i) it uses its own amino-terminal domain as a protein primer for initiation of DNA synthesis, (ii) it cannot be isolated in an active form from virions without partial proteolysis or denaturation, and (iii) under normal circumstances it is active only on the endogenous pregenomic RNA that is encapsidated with it in the viral cores. The mechanism of this template commitment is unknown, although the covalent linkage between P and the viral DNA is likely to contribute to it. RNAseH cleaves the RNA strand in a RNA:DNA heteroduplex, but it does not cleave DNA or RNA that is not hybridized to DNA. The RNAseH activity of P is required to remove the RNA pregenome after it has been copied into DNA during reverse transcription. If there is no RNAseH activity, positive polarity DNA cannot be synthesized and no infectious virions are produced. The hepadnaviral RNAseH activity has not yet been directly measured in viral core particles, however, its existence is inferred from four observations, (i) Sequence alignment of P with known RNAseH enzymes reveals conserved motifs in P. (ii) Mutation of the putative RNAseH active site residues in both Hepatitis B Virus (HBV) and duck hepatitis B virus (DHBV) P blocks synthesis of mature hepadnaviral DNA and results in the expected RNA:DNA heteroduplex. (iii) RNAseH activity has been detected for the human hepatitis B virus when the RNAseH domain was expressed as a fusion with the maltose binding protein. (iv) RNAseH activity has been detected in activity gels employing purified DHBV cores, but the apparent molecular weight of the RNAseH was less than that of the native DHBV P, indicating that P was either cleaved prior to the assay, or that the signal came from a cellular RNAseH. In this study we attempted to directly detect the RNAseH activity of DHBV P within viral cores employing exogenous

substrates. Extensive efforts employing a wide range of conditions and substrates yielded no DHBV RNAseH activity. Because genetic and biochemical analyses indicate that P possesses an intrinsic RNAseH activity, we conclude that the hepadnaviral RNAseH activity is likely to be "substrate committed" in a manner similar to the template commitment observed for its DNA polymerase activity.

CONCLUSION

Conclusions DHBV RNAseH activity could be detected on RNA:DNA heteroduplexes synthesized within DHBV core particles in the process of viral reverse transcription, but no RNAseH activity could be detected exogenously provided RNA:DNA heteroduplexes. Extensive controls lead us to believe that the RNAseH active site is most probably "substrate committed" in a manner similar to the "template commitment" of the reverse transcriptase activity. We acknowledge that we have not formally proven this conclusion, but at a minimum, we have demonstrated that the DHBV RNAseH activity cannot degrade exogenous substrates under a very wide variety of conditions that support vigorous activity by the associated DNA polymerase domain.