

High affinity binding of proteins HMG1 and HMG2 to semicatenated DNA loops

ABSTRACT

Among all the DNA structures that have been described so far, HMG1 and HMG2 interact with Form X, which has a base of semicatenated DNA junction to give it the highest affinity of over 4 orders of magnitude for each structure. This implies that if there are comparable structures in the cell nucleus, one of the functions of these proteins may be associated with the exceptional quality of DNA hemicatenanes to establish a stable and irreversible association between two remote regions of interest within the genome.

INTRODUCTION

Term-enhanced Hepadnaviruses are small, enveloped hepatotropic viruses that replicate by reverse transcription of a pregenomic RNA molecule. P binds to the 5' copy of and commences DNA synthesis, using a tyrosine residue near its own amino-terminus as supplementary information. DNA synthesis occurs after 3–4 nucleotides, and then the protein-linked minus-strand DNA is transferred to this copy (the doppelganger) within the 3' terminal duplication. The minus-strand DNA is prepared as a primer by the pregenomic RNA, and its ribonucleaseH activity degrades it during synthesis. In spite of this, the RNaseH activity fails to digest the extreme 5' end of the template ARN, and the fragment is then transferred to direct repeat 2 (DR2), where it serves as a primer for synthesising plus-strand DNA. Unlike the reverse transcriptases of retroviruses that are better understood, the hepadnaviral P protein differs in several ways: it starts DNA synthesis with its amino-terminal domain, cannot be obtained from virions without partial proteolysis or denaturation, and is only active on the endogenous pregenomic RNA that is encapsulated in the viral cores. It is not known how this cleaved out but appears due to the covalent linkage between P and the viral DNA, which is likely to explain why: RNaseH inserts the end of the ribonuclear layer in a RNA:DNA heteroduplex, but it does no damage any DNA or non-hybrid material (in that case the protein P doesn't cut the part of its own end after it had already been reverse transcriptionally copied into DNA). The absence of RNaseH activity hinders the production of infectious virions or positive polarity DNA. Although not yet measured directly in viral core particles, its presence is suggested by four observations, (i) Sequence alignment of P with known RNaseH enzymes shows conservation motifs in P. By modifying their active site residues, the expected RNA:DNA heteroduplex is prevented from being synthesized by blocking mutations in both Hepatitis B Virus (HBV) and duck hepatitis B virus (DHBV) P. The detection of RNaseH activity for the human Hepatitis B virus was confirmed through the expression of an ion called fusion with the maltose binding protein. Purified DHBV cores were used to generate activity gels, but the apparent molecular weight of RNaseH was lower than that of the native PH, suggesting that P was either cleaved or signaled by a cellular RNaseH. We attempted to directly detect the RNaseH activity of DHBV P within viral cores using substrates from out of the virus. However, no specific results were obtained despite extensive experiments with different conditions and substrate combinations. Given that P has intrinsic RNaseH activity, we hypothesize that the hepadnaviral RNaseH activation will be "substrate committed" in a similar way to the template commitment observed for its DNA polymerase activity.

CONCLUSION

A DNA loop with its base maintained by a semicatenated DNA junction has been identified as Form X, which was previously observed through polyacrylamide gel electrophoresis of DNA fragments containing traces of the CA microsatellite poly (CA) poly(TG) but had not been characterised. Hemicenanes, which are structures that contain DNA, had not been previously suggested to exist in the cell. The ability to prepare these structures, along with their remarkable stability, should allow for studying their evolution and potential function when introduced to living cells.