Casein kinase II and protein kinase C modulate hepatitis delta virus RNA replication but not empty viral particle assembly

葉添順
Yeh;T.-S.;S. J. Lo;P.-J. Chen;and Y.-H. W. Lee.

Abstract

Hepatitis delta virus (HDV) contains two virus-specific delta antigens (HDAgs), large and small forms, which are identical in sequence except that the large one contains 19 extra amino acids at the C terminus. HDAgs are nuclear phosphoproteins with distinct biological functions; the small form activates HDV RNA replication, whereas the large form suppresses this process but is required for viral particle assembly. In this study, we have characterized the phosphorylative property of HDAg in a human hepatoma cell line (HuH-7) and examined the role of phosphorylation in HDAg function. As demonstrated by in vivo labeling and kinase inhibitor experiments, the phosphorylation levels of both HDAgs were diminished by the inhibitor of casein kinase II (CKII). Nevertheless, phosphorylation of only the small form could be markedly reduced by the protein kinase C (PKC) inhibitor, suggesting different phosphorylation properties between the two HDAgs. When these two kinase inhibitors were added separately to the transient-expression system, HDV RNA replication was profoundly suppressed. In contrast, the inhibitors did not affect the assembly of empty HDAg particle from HDAgs and hepatitis B virus surface antigen. To further examine the role of phosphorylation in HDAg function, two conservative CKII recognition sites at Ser-2 and Ser-123 of both HDAgs and one potential PKC recognition site at Ser-210 of the large HDAg were altered to alanine by site-directed mutagenesis. Transfection experiments indicated that mutation at Ser-2, but not Ser-123, significantly impaired the activity of the small HDAg in assisting HDV RNA replication. This property is in accordance with our observation that Ser-2, not Ser-123, was the predominant CKII phosphorylation site in the small HDAg. Our studies also excluded the possibility that the phosphorylation of Ser-2, Ser-123, or Ser-210, had roles in the trans- suppression activity of the large HDAg, in the assembly of empty virus- like HDAg particle, and in the nuclear transport of HDAgs. In conclusion, our results indicate that both CKII and PKC positively modulate HDV RNA replication but not the assembly of empty HDAg particle. The role of CKII in HDV replication may at least in part be accounted for by the phosphorylation of Ser-2 in the small HDAg. The effect of PKC on HDV RNA replication is, however, not to mediate the phosphorylation of the conservative Ser-210 in the large HDAg but rather to act on as-yet-unidentified Ser or Thr residues in the small HDAg or cellular factors. These findings provide the first insight into the roles of phosphorylation of the two HDAgs in the HDV replication cycle.