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The virion N protein of infectious bronchitis virus is more phosphorylated than the N protein from infected cell lysates

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Abstract

Because phosphorylation of the infectious bronchitis virus (IBV) nucleocapsid protein (N) may regulate its multiple roles in viral replication, the dynamics of N phosphorylation were examined. ^{32}P-orthophosphate labeling and Western blot analyses confirmed that N was the only viral protein that was phosphorylated. Pulse labeling with ^{32}P-orthophosphate indicated that the IBV N protein was phosphorylated in the virion, as well as at all times during infection in either chicken embryo kidney cells or Vero cells. Pulse-chase analyses followed by immunoprecipitation of IBV N proteins using rabbit anti-IBV N polyclonal antibody demonstrated that the phosphate on the N protein was stable for at least 1 h. Simultaneous labeling with ^{32}P-orthophosphate and ^{3}H-leucine identified a 3.5-fold increase in the ^{32}P:^{3}H counts per minute (cpm) ratio of N in the virion as compared to the ^{32}P:^{3}H cpm ratio of N in the cell lysates from chicken embryo kidney cells, whereas in Vero cells the ^{32}P:^{3}H cpm ratio of N from the virion was 10.5-fold greater than the ^{32}P:^{3}H cpm ratio of N from the cell lysates. These studies are consistent with the phosphorylation of the IBV N playing a role in assembly or maturation of the viral particle.

Keywords: IBV; Pulse-chase; Phosphorylation; Nucleocapsid protein; Double labeling

Introduction

Infectious bronchitis virus (IBV) is a prototype of the Coronaviridae family (Collisson et al., 1992). Coronaviruses are positive-stranded RNA viruses that primarily cause respiratory and enteric infections. Infection with highly contagious IBV causes respiratory disease in chickens resulting in high mortality in young chicks. It also affects the enteric, reproductive, and urinary systems (Alexander and Gouch, 1978; Crinion and Hofstad, 1972).

IBV has four structural proteins, the membrane (M), spike (S), nucleocapsid (N), and small membrane associated (E) proteins. Similar to other coronavirus N proteins, the IBV N is a basic, phosphorylated protein. The 409 amino acid IBV N has a highly conserved region between residues 238 and 293 (Hogue and Brian, 1986; Wilbur et al., 1986; Williams et al., 1992). The N protein directly binds viral genomic RNA and forms a helical ribonucleoprotein complex (RNP) (Davies et al., 1981). It has been implicated in playing a role in genome replication, transcription of subgenomic RNA, translation, and formation of the nucleocapsid (Masters and Sturman, 1990; Tahara et al., 1998).

Murine hepatitis virus (MHV) N protein, the most abundant viral protein, has been reported to be synthesized almost 4–5 h post-infection (p.i.) in Sac (−) cells infected at a high multiplicity of infection (M.O.I.) (Siddell et al., 1980). In MHV, a 57-kDa (p57) non-phosphorylated form is the precursor to the 60-kDa (p60) phosphorylated form of the protein. The p57 is present only in the cytosol, whereas p60 is also found associated with membranes (Stohlman et al., 1983).

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Analysis of the tryptic peptides of the ^{32}P-labeled N proteins of two plaque morphology variants of the neurovirulent JHM strain of MHV and the non-neurovirulent prototype strain A59 indicated that the N protein of MHV strains differ in their sites of phosphorylation (Wilbur et al., 1986). No differences were detected in the tryptic peptide maps of the MHV N protein purified from the virion, the infected cytosol and the infected cellular membranes (Wilbur et al., 1986). Although two phosphorylated forms of the N protein, 50 kDa and 55 kDa, were detected in bovine coronavirus (BCoV)-infected cells, only the 50-kDa form was detected in the virion. The 55-kDa form was susceptible to calf intestinal alkaline phosphatase (CIAP) treatment, while the 50-kDa form was not susceptible to CIAP treatment (Hogue, 1995; King and Brian, 1982).

Whereas the IBV N protein has been shown to be phosphorylated in infected cells (Lomniczi and Morser, 1981), the current study demonstrates that the N protein is phosphorylated at all times in cell lysates, as well as in the virion. We also show that the phosphorylation of N is greater in the IBV viral particle.

Results

N from infected cell lysates and the virion differ in electrophoretic mobility

The N protein is the only coronavirus structural protein known to be phosphorylated, with the exception of the M protein in SARS CoV which has recently been shown by mass spectrometry to have a unique phosphorylation site. (Siddell, 1995; Zeng et al., 2004). Because phosphorylation is known to play critical roles in the function of proteins, the nature of the phosphorylation of N was compared in the cell at varying times p.i. and in the supernatant virion. At 8 h p.i., and 16 h p.i., ^{32}P-orthophosphate ($^{32}P_i$)-labeled IBV N protein was purified by immunoprecipitation of infected Vero cell lysates and from supernatant viral particles, respectively, using rabbit anti-N polyclonal antibody. The N protein from both the intracellular lysates and virions were found to be phosphorylated (Fig. 1A). Two phosphorylated forms of the cell lysate N were detected, as distinguished by electrophoretic mobilities (Fig. 1A). Using Western blot analysis, both bands reacted with chicken anti-

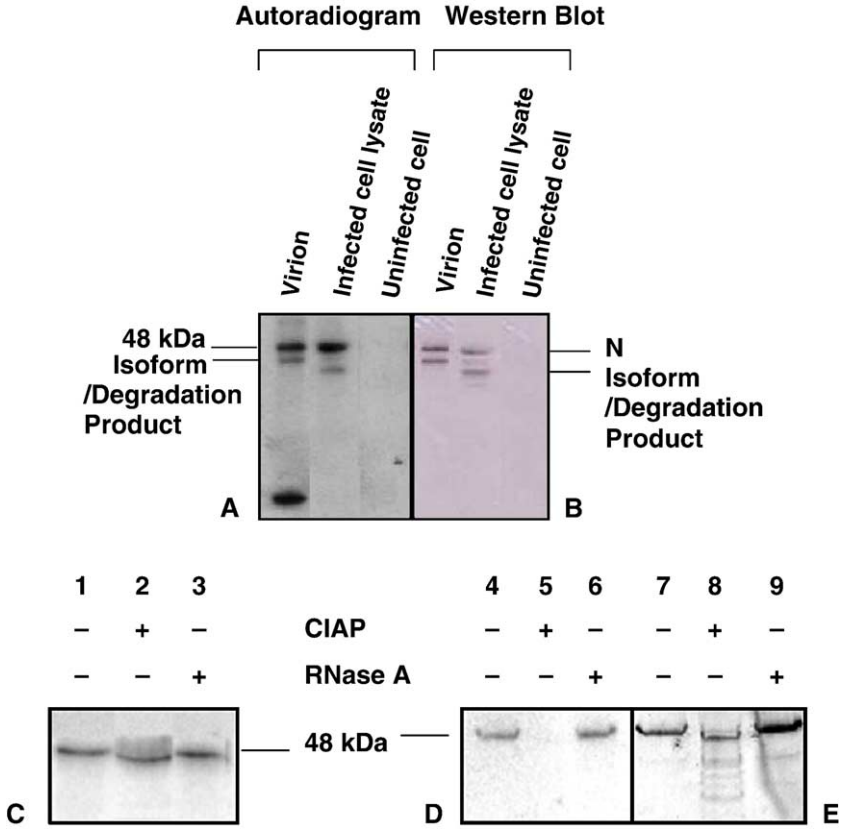

Fig. 1. Calf intestinal alkaline phosphatase treatment (CIAP) of the IBV N protein. ^{32}P-orthophosphate-labeled IBV N protein from lysates of infected Vero cells and from virions was immunoprecipitated with rabbit anti-IBV polyclonal antibody before separating by SDS–PAGE. Panel A represents the autoradiogram and panel B, the Western blot using chicken anti-IBV (Gray) polyclonal antibody. The labeled N protein was treated with 20 U CIAP and resolved by denaturing electrophoresis in a 15% polyacrylamide gel. Panel C represents an autoradiogram of the virion N. Panels D and E show the autoradiogram and Western blot, respectively, of cellular N obtained 8 h p.i.