Analysis of hemaggulutinating ability of the rabbit hemorrhagic disease virus (RHDV) capsid protein expressed in *Escherichia coli*

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Rabbit hemorrhagic disease virus (RHDV) belonging to the family Caliciviridae causes fatal necrotizing hepatitis and disseminated intravascular coagulation (DIC) in adult rabbits.

Hemaggulutination (HA) tests using infected rabbit liver homogenate and human 'O' erythrocytes have been established as a simple and rapid diagnostic method. However, some non-hemagglutinating viruses were isolated from Rainham, UK in 1993 (Rainham 93), Ireland in 1995 (Ireland 95a/95b) and Japan in 1995 (Japan 95). This alteration of the hemagglutinating activity was correlated with a few amino acid changes in their capsid protein (VP60) sequences.

The nucleotide sequence of Korea 91 VP60, one of the hemagglutinating isolates, was determined. Comparison of the deduced amino acid sequence of Korea 91 VP60 with that of a German isolate (Germany 88) revealed that the amino acid changes in the VP60 of Korea91 were scattered thoughout VP60. On the other hand, between those of Japan 95 and Germany 88, amino acid changes were confined to the hypervariable region. Importantly, in the hypervariable region, three unique amino acid changes that did not exist in the hemagglutinating isolates were commonly observed in non-hemagglutinating isolates, including the Japanese one and two Irish isolates. These results suggest that the amino acid substitutions in the hypervariable region of VP60 may influence the hemagglutinating activities.

The VP60 genes of hemagglutinating Korea 91 and non-hemagglutinating Japan 95 were subcloned and overexpressed in *E. coli* as 70 KDa fusion proteins. HA tests were performed using the purified recombinant VP60 and human type O and B erythrocytes. Both of the recombinant VP60s hemagglutinated type O and B erythrocytes at 4°C. In contrast, at 20°C, Japanese VP60 did not hemagglutinate either type of erythrocyte, whereas Korean VP60 agglutinated type B erythrocytes.

Although *E. coli* expression systems have been widely used to analyse the properties of RHDV, there has been no report concerning its HA activity. In the present study, VP60 of Korea91 and Japan95 expressed in *E. coli* showed hemagglutinating activities. In addition, the hemagglutinating activity of recombinant VP60 was different between isolates. These data suggest that the hemagglutinating ability of RHDV is associated with amino acid sequences of a few specific sites on the VP60 molecule.