Cleavage Site between VP1 and P2A of Human Rhinovirus Is Different in Serotypes 2 and 14

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SUMMARY

The viral capsid protein VP1 of human rhinovirus serotype 2 (HRV2) was cleaved with cyanogen bromide. The peptides thus obtained were separated on an HPLC butyl reversed phase column. Their positions on VP1 were determined by amino-terminal sequencing using the known nucleotide sequence of the genomic RNA of HRV2. The putative carboxy-terminal peptide was further cleaved with trypsin and the resulting fragments were separated on a C18 reversed phase column. Amino-terminal sequencing of the C-terminal peptide revealed alanine as being the carboxy terminus of VP1 in HRV2. This indicates that the processing of the polyprotein is different in HRV2 from the processing previously reported for HRV14 and poliovirus.

Picornaviruses contain a genomic RNA of about 7400 nucleotides with positive polarity which is translated into a polyprotein of about 240K (for review, see Agol, 1980). Under normal conditions this protein is never observed as it is processed during translation by two viral proteases in an autocatalytic manner (Nicklin et al., 1986). In the case of poliovirus amino- and carboxy-terminal sequences of several viral proteins have been determined (Kitamura et al., 1981; Semler et al., 1981; Emini et al., 1982; Pallansch et al., 1984). The knowledge of the gene order together with biochemical and genetic data (Hanecak et al., 1982, 1984; Toyoda et al., 1986) revealed that P3C and P2A act as proteases with different cleavage specificities (for nomenclature, see Rueckert & Wimmer, 1984). Similar proteases have been demonstrated for encephalomyocarditis virus (Palmenberg et al., 1984) and for foot-and-mouth disease virus (Burroughs et al., 1984). Due to the high sequence homology of the human rhinoviruses HRV2, HRV14 and HRV89 with poliovirus it is most likely that the same proteases exist in rhinoviruses and that the processing of the polyprotein is similar. Some of the cleavage sites have been identified by amino-terminal sequencing of the capsid proteins VP1, VP2 and VP3 of HRV2 and HRV89 (Skern et al., 1985; Duechler et al., 1987). For HRV14 the proposed carboxy termini of the capsid proteins could be confirmed on the electron density map obtained by X-ray crystallography (Stanway et al., 1984; Callahan et al., 1985; Rossmann et al., 1985).

Examination of the poliovirus polyprotein for characteristics common to the cleavage sites of each of the two proteases showed that P3C cleaves between Gln-Gly and P2A cleaves between Tyr-Gly (Toyoda et al., 1986). This specificity is strictly adhered to in poliovirus whereas Glu-Ser, Gln-Asn, Glu-Gly, Gln-Ser and Gln-Ala pairs are apparently also processed by P3C of rhinoviruses. The amino acid pair Tyr-Gly between VP1 and P2A which is cleaved by P2A is conserved in HRV14 and poliovirus. Based on these data the cleavage carried out by P2A in HRV2 was postulated to be between Tyr-Val or Tyr-Ser (Skern et al., 1985) or Phe-Val in HRV89 (Duechler et al., 1987). The comparison of the three-dimensional structure of HRV14 and the model of HRV2 derived from it showed that the eight carboxy-terminal amino acids of VP1 in HRV2 (assuming Tyr 289 as carboxy terminus) were difficult to accommodate in the
model (Blaas et al., 1987). A folding pattern in this region similar to the structure of HRV14 would thus have been possible only if VP1 were about eight amino acids shorter than originally proposed. This would, however, require a different amino acid pair to be cleaved during the processing of the growing polypeptide chain in HRV2. The present study was undertaken to determine the carboxy-terminal amino acids of VP1 in HRV2 and thus to gain more insight into the signals utilized in the course of viral maturation.

Two mg purified HRV2 was dissociated in sample buffer and run on a 10% polyacrylamide gel in the presence of SDS (Laemmli, 1970). The viral protein VP1 was eluted (Skern et al., 1985) and cleaved with a 100-fold molar excess of CNBr. The fragments were separated on a Bakerbond butyl wide-pore reversed phase HPLC column run with a gradient of 0.1% trifluoroacetic acid (TFA) to 80% acetonitrile–0.1% TFA. From the amino acid sequence as deduced from the known nucleotide sequence of the genomic RNA of HRV2 (Skern et al., 1985) it can be inferred that nine fragments should be obtained upon CNBr treatment if tyrosine 289 were the correct carboxy terminus (Fig. 1). The major peaks obtained from the butyl column were subjected to amino-terminal sequencing on a AB-470A gas phase sequencer (Applied Biosystems, Foster City, Ca., U.S.A.). Fig. 1 shows the amino acid sequences thus determined on top of VP1 as derived from the RNA sequence. Two peaks each contained single peptides, and one peak was found to contain two peptides (CN7 and CN8) since each sequencing step resulted in the identification of two amino acids. From these data CN1, CN5, CN7 and CN8 could be mapped on the sequence of VP1. In all cases the amino acid sequence derived from the cloned DNA was confirmed.

Since CNBr cleaves after methionine all the resulting fragments except the C-terminal one contain homoserine derived from the methionine. VP1 was therefore prepared from L-[2(n)-3H]methionine-labelled virus and was cleaved with CNBr. If VP1 ended before methionine 288, peptide CN8 of VP1 should be the only one without label (Fig. 1). Therefore the fractions eluted from the butyl reversed phase column containing the two peptides CN7 and CN8 were pooled and 1/100 (v/v) was separated on a high resolution polyacrylamide gel in the presence of SDS (Swank & Munkres, 1971). After staining with silver (Merill et al., 1981) the lane containing CN7 and CN8 was cut into 2 mm slices perpendicular to the direction of migration and the radioactivity of each slice was determined in a Packard sample oxidizer (Fig. 2). At the position corresponding to CN7 (2.9K calculated molecular weight) a peak of radioactivity was found whereas the band migrating behind it and thus corresponding to CN8 (6K) was not labelled, indicating that CN8 contained the carboxy terminus of VP1 (Fig. 2). These two peptides were then separated on a Waters ProteinPack 125 column with 0.1% TFA as eluent.
**Short communication**

Fig. 2. Separation of CNBr fragments CN7 and CN8 on a 12.5% polyacrylamide gel (Swank & Munkres, 1971). The stained gel (a) has the positions of peptides and their molecular weights displayed. The gel was cut into 33 slices; the radioactivity in each gel slice was determined in a Packard sample oxidizer and is shown in (b). Migration was from left to right.

CN8 was digested with trypsin at 2% (w/w) with respect to the amount of peptide and the fragments were separated on a μ Bondapack C18 reversed phase column using a gradient of 0.1% TFA to 60% acetonitrile–0.1% TFA. Assuming that trypsin did not cleave the two Arg-Pro bonds under these conditions (Smyth, 1967) the C-terminal peptide was expected to contain more than 10 amino acids and thus to be the longest peptide in the digest (see Fig. 1). Moreover a calculation of the retention times predicted that this peptide would be eluted after the others provided that it contained more than 11 amino acids (Browne et al., 1982). T7 was therefore chosen to be sequenced first and was found to contain the sequence of 15 amino acids as indicated on Fig. 1. The last amino acid detected on the sequencer was Ala 283 which thus represents the carboxy terminus of VP1.

In contrast to P3C, which is responsible for at least nine cleavages of the polyprotein, P2A carries out only one cleavage with high efficiency between VP1 and itself. The amino acid pair at this position is Tyr-Gly in poliovirus and HRV14 whereas no such sequence exists at the boundary between VP1 and P2A in HRV2, HRV89 and coxsackieviruses B1 and B3 (Lindberg et al., 1987; Iizuka et al., 1987). There is, however, a site in P3D which is thought to be processed by P2A as well, although less frequently, giving rise to P3C' and P3D' in poliovirus (Toyoda et al., 1986). The same Tyr-Gly pair can also be found in the sequences of coxsackieviruses, HRV2, HRV14 and HRV89. Moreover proteins probably produced by this cleavage reaction have been demonstrated for HRV1A (McLean et al., 1976). It is thus striking that an amino acid pair with rather different properties is utilized as a cleavage site between VP1 and P2A in HRV2. Furthermore an Ala-Gly pair is missing at a similar position in coxsackieviruses B1 and B3 and in HRV89, indicating that yet another processing site must be used in these viruses. This would point to a generally low specificity of P2A for a certain amino acid pair or sequence which is, however, in contrast with the low number of cleavages observed. Taken together, these findings suggest a much stronger dependence of P2A on the folding of the polypeptide chain than on the particular amino acid pair which is cleaved. Predictions of cleavage sites in other related picornaviruses can thus be based only on homology considerations taking into account the vicinity of the presumed cleavage site.
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REFERENCES


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