The Location of the Bromelain Cleavage Site in a Hong Kong Influenza Virus Haemagglutinin

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SUMMARY

The site of bromelain cleavage in the haemagglutinin of the Hong Kong influenza virus A/Memphis/102/72 has been determined by using a diagonal peptide mapping procedure on the thermolytic digest of amidated BHA. The data show that bromelain cleavage removes the C-terminal 46 residues from HA2, and that the new carboxyl-terminal residue of BHA2 is Gly 175. This is close to the beginning of the hydrophobic membrane-interacting sequence that starts at residue 183.

It has been known for several years that the haemagglutinin of influenza virus can be cleaved off the virus coat by incubation of whole virus with bromelain (Compans et al., 1970), trypsin or chymotrypsin (Schulze, 1970). The truncated haemagglutinin (BHA) obtained by bromelain digestion of whole virus is antigenically (Brand & Skehel, 1972; Wrigley et al., 1977) and structurally (Flanagan & Skehel, 1977; Wiley et al., 1977) intact and has been prepared routinely for sequencing purposes (Skehel & Waterfield, 1975; Waterfield et al., 1979) and X-ray crystallography (Wiley & Skehel, 1977; Wilson et al., 1980) because of its high solubility.

Despite the wealth of structural knowledge about the haemagglutinin molecule, there is no precise information available regarding the cleavage site. Skehel & Waterfield (1975) showed that bromelain left the HA1 chain unaffected, but removed the hydrophobic ‘tail’ of the HA2 chain. Amino acid analyses suggested that 50 to 90 residues were removed from the carboxyl-terminal part of the HA2 chain (Skehel & Waterfield, 1975; Waterfield et al., 1979).

In this paper we report on the site of bromelain cleavage in the Hong Kong haemagglutinin from A/Memphis/102/72. We have located the new carboxyl-terminal residue of bromelain-cleaved HA2 by a diagonal peptide mapping procedure first described by Duggleby & Kaplan (1975). This modification procedure converts protein carboxyl groups, including the carboxyl terminus, into uncharged amides. On subsequent digestion of the modified protein all peptides generated will have free carboxyl groups except the carboxyl-terminal peptide which will be amidated and can be isolated by the diagonal procedure.

HA was isolated by electrophoresis on cellulose acetate blocks as described by Ward & Dopheide (1980), dialysed extensively against 0.01 M-NH₄HCO₃ and lyophilized. HA (10 mg) was digested with bromelain (2.5 mg) in 6 ml, containing 0.01 M-tris-HCl pH 7.4, 0.01 M-mercaptoethanol and 0.02 M-EDTA for 8 h at 37 °C. The digest was fractionated by centrifugation on a sucrose gradient (Brand & Skehel, 1972).

BHA (4 mg, 50 nmol) was amidated in saturated guanidine-HCl (1 ml) containing ethanolamine (20 µl, 300 µmol) and adjusted to pH 4.7 with 1 M-HCl from a microsyringe. 1-Ethyl-3(3-diaminopropyl)carbodiimide (15 mg, 80 µmol) was then added with stirring, and the pH of the solution maintained at 4.7 for 6 h. After 25 h dialysis against 0.01 M-NH₄HCO₃, the modified protein was digested with thermolysin (200 µg, 16 h at 37 °C); the soluble fraction of the digest was fractionated by two-dimensional paper electrophoresis, initially at pH 6.5 then at pH 1.9, using taurine and valine amide as markers. The amidated carboxyl-terminal peptides should lie on a diagonal between these markers. Peptides were visualized with floroescamine (Mendez & Lai, 1975).
In Fig. 1 we show the location of fluorescent spots, which were then eluted, hydrolysed and analysed. Peptide 1 contained only traces of peptide material while peptides 3, 4 and 5 were all derived from the carboxyl-terminal part of HA1. Peptide 2 contained equal amounts of isoleucine, glycine, lysine and ethanolamine, and its mobility agreed with that expected of a peptide with a mol. wt. of 360 (Ile 1, Gly 1, Lys 1, ethanolamine 1) and two net positive charges (Offord, 1966). From the specificity of thermolysin cleavage, isoleucine is amino-terminal, which was confirmed by dansylation. The only peptide with the sequence Ile-Lys-Gly occurs at residues 173 to 175 of HA2 (Fig. 2), and the occurrence of glycine as the carboxyl-terminal residue is in good agreement with the known specificity of bromelain (Enzyme nomenclature, 1978). These findings indicate that the new carboxyl-terminal residue generated by bromelain digestion of A/Mem/102/72 (H3) HA is Gly 175 of the HA2 chain, and this is consistent with other data. In the course of a structural study on Mem/72 HA2, Dopheide & Ward (1980) found that a very hydrophobic sequence commenced after residue 183. Since this sequence is presumed to be involved in membrane interaction, the bromelain cleavage should be N-terminal to it.

Also, McAuley et al. (1978) suggested that the carboxyl-terminal cyanogen bromide peptide of A/Jap/57 BHA2 contained approx. 25 residues, and homology with the Mem/72 HA2 structure (Ward & Dopheide, 1979) and the recently completed Jap/57 HA2 sequence (Gething et al., 1980) show that this peptide starts at residue 150. This would place the bromelain cleavage site near residue 174. Also, Skehel & Waterfield (1975) and Waterfield et al. (1979) indicated that bromelain digestion of Bel HA (H10) removed 50 to 90 residues off HA2, from which a final size of BHA2 of 130 to 170 can be calculated. The finding of only one single HA2-related peptide is surprising, in view of both the wide specificity of bromelain, and the report of McAuley et al. (1978) that the carboxyl-terminal CNBr peptide from Jap/57 BHA2 was heterogeneous. This heterogeneity, however, would have been caused by contamination with peptides resulting from partial cleavage by CNBr, rather than by multiple cleavage by bromelain.

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Fig. 2. The sequence of Memphis/72 HA2 (Sleigh et al., 1980; Dopheide & Ward, 1980). The bromelain cleavage site is indicated by a vertical arrow, while the isolated carboxyl-terminal peptide is underlined.
Short communications

REFERENCES


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