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**Porphyromonas gingivalis** RgpA and Kgp Proteinases and Adhesins Are C Terminally Processed by the Carboxypeptidase CPG70

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**Porphyromonas gingivalis** is a bacterial pathogen that produces the polyproteins RgpA and Kgp, which are proteolytically processed into proteinases and adhesins. We have demonstrated that the RgpA and Kgp proteinases and adhesins are C terminally processed by carboxypeptidase CPG70 by sequencing C-terminal peptides from both the wild type and an isogenic CPG70 mutant, using ion trap mass spectrometry.

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The mass spectrum obtained for RgpA15/Kgp15 from the wild type contained two major peaks, the one at \( m/z \) 2083 previously having been assigned to the processed C-terminal peptide (7). In the spectrum obtained for RgpA15/Kgp15 from the mutant, however, this peak was replaced by a peak 128 Da higher at \( m/z \) 2211, consistent with the presence of an additional Lys residue (data not shown). To provide direct evidence that these peaks at \( m/z \) 2083 and 2211 are the processed and unprocessed C-terminal peptides, respectively, the corresponding peptide digests were desalted and concentrated using \( \mu \)C18 Zip Tips (Millipore), following the manufacturer’s instructions, and 2 \( \mu \)l of eluate was pipetted into a nanospray needle (Econo12 PicoTip; New Objective) and analyzed on an Esquire LC ion trap mass spectrometer fitted with a nanospray source (Bruker Daltonics, Bremen, Germany) (Fig. 3). The capillary voltage was set to 600 V, and the drying gas (\( N_2 \)) was set to 2 liters/min and 50°C. The trap drive, skim 1, and octopole voltages were optimized prior to each MS/MS analysis. Strong signals corresponding to the doubly charged forms of these peptides were observed and selected for MS/MS analyses. The identities of the peptides were determined by performing an MS/MS ion search against the National Center for Biotechnology Information database. A single, highly significant hit was obtained for both processed (Fig. 3A) and unprocessed (Fig. 3B) forms of the RgpA15/Kgp15 C-terminal peptide. The MS/MS spectra exhibited excellent sequence coverage, enabling the detection of a modified peptide in each spectrum. The modified peptides are the major forms present and contain a deamidated Asn residue, as indicated by “\( N^* \)” in Fig. 3.

Using the same technique, the processed and unprocessed forms of the C-terminal peptides of RgpA45, Kgp48, RgpA17, and Kgp14 were identified (Table 1), indicating that the same carboxypeptidase, CPG70, was responsible for the removal of C-terminal Lys in each domain. The C-terminal peptides of RgpA44 and Kgp39 are identical and have average masses (MH−) of 4,619 and 4,747 Da for the processed and unprocessed forms, respectively. The large size of these made them difficult to analyze by ion trap MS; however, peaks at these masses were observed by matrix-assisted laser desorption ionization MS and were specific to domains derived from the wild-type and mutant strains, respectively (data not shown).
Having shown that CPG70 is involved in processing of RgpA and Kgp, an important question that arises is the relevance of this processing to virulence. In addition to the possibility that CPG70 has a direct role in virulence by processing host factors, it is also possible that the role of CPG70 in virulence is linked to Kgp in their dual role of C-terminal processing. One possibility is that the removal of C-terminal lysine is required to enable the various adhesin and proteolytic domains of both RgpA and Kgp to correctly associate and form a virulent conformation. Processed RgpA15/Kgp15 has a theoretical mass of 12.8 kDa. This adhesin migrated on the 2D-PAGE gel, however, at approximately 18 kDa (Fig. 2A, spot 3). In comparison, under the same experimental conditions, unprocessed RgpA15/Kgp15 was found to migrate to a lower molecular mass (Fig. 2B, spot 3), suggesting that the presence of C-terminal Lys has an effect on the structure of this domain. Whether the complex of RgpA and Kgp domains with unprocessed C-terminal Lys residues is less virulent, however, remains to be established. In conclusion, we have shown that CPG70 is involved in the C-terminal processing of the RgpA and Kgp polyproteins.

**REFERENCES**