[7]. Further, P-III SVMPs are major
ts of rear-fanged snake venoms and account for several of the
effects observed following envenomation [11,12]. However, to
are few studies on the primary structure of P-III SVMPs found
ged snake venoms. Moreover, there are no published studies
utocatalytic processing, either in vivo or in vitro.

nfibrase is a 57.5-kDa metalloproteinase isolated from the
Philodryas patagoniensis (Patagonia Green Racer), a South
rear-fanged snake which is now considered a member of the
sadidae [13]

enzyme and its main autoproteolytic fragment, we demonstrated that it is subjected to specific autoproteolytic cleavage at the initiation of the disintegrin domain.

(data not shown), indicating that it is still connected by disulfide

band of ~32.6 kDa (Fig. 1B), whose molecular mass is compatible with disintegrin-like/cysteine-rich domains [18]. This fragment is not observed when non-reduced samples were submitted to SDS-PAGE

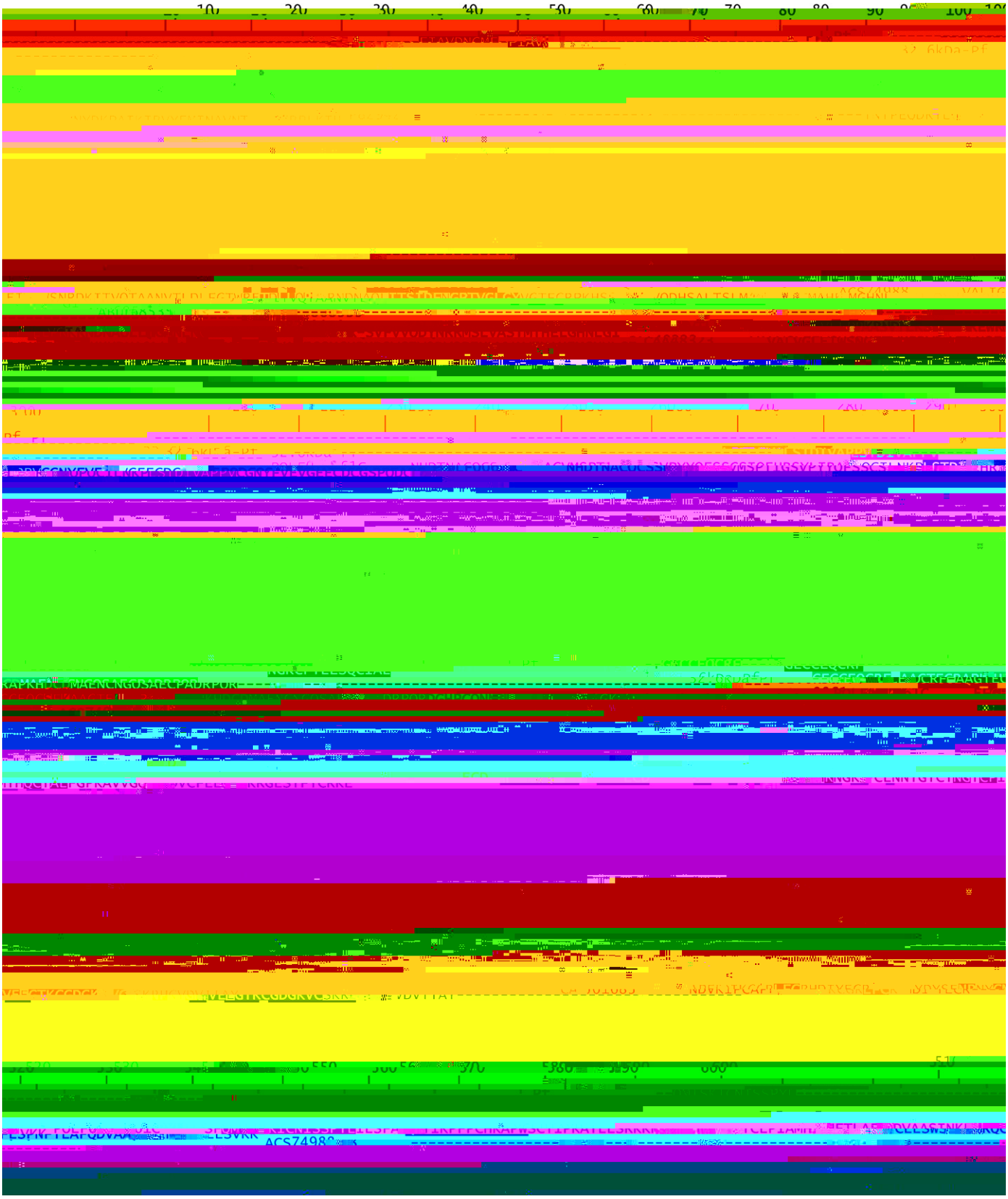

Fig. 3. Sequence alignment of the N-terminus of the 32.6-kDa autoproteolytic fragment of patagonfibrase (red), and peptides sequenced by LC-MS/MS (see Table 1) from patagonfibrase and its 32.6-kDa autoproteolytic fragment, with other P-III SVMPs. Protein sequences were aligned using the program ClustalW [34]. The numbers indicate the amino acid residues of a metalloprotease from P. olfersii, POLF0061C [11]. The other SVMPs were referenced by their GenBank accession numbers: ACS74988, from P. olfersii (Dipsadidae); ABU68535, from Thrasops jacksoni (Colubridae); P82942, kaouthiagin, from Naja kaouthia (Elapidae); CAA48323, jararhagin, from Bothrops jararaca (Viperidae); and CAJ01683, from Echis ocellatus (Viperidae). Identical residues are boxed in black. The boundary between the metalloproteinase and disintegrin domains of SVMPs is indicated, and disintegrin-like sequences are shown in green.

parent structure play an important role in stabilizing and tightening the segment connecting the proteolytic domain with the succeeding disintegrin domain [19,22]. As shown here, patagonfibrase structure is partially stabilized by Ca^{2+}, since there is a proteolysis product at 52.2 kDa in the presence of this ion. This fragment likely represents patagonfibrase processed to release a portion of the amino-terminal

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