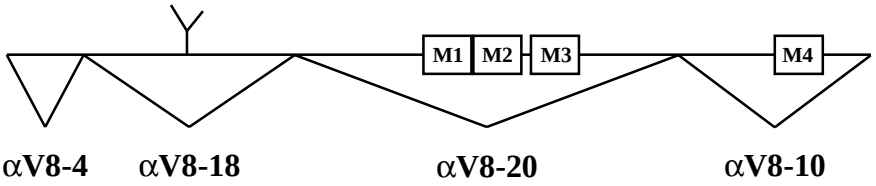
M1
M2
M3
M4
αV8-4
αV8-18
αV8-20
αV8-10

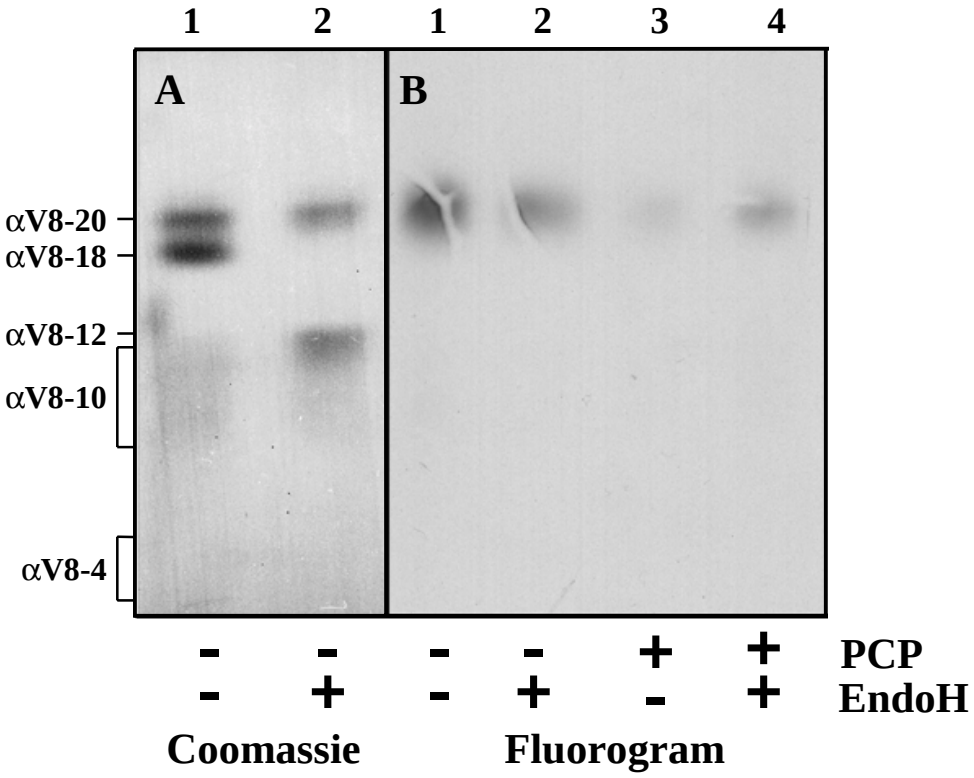
1
2
1
2
3
4
A
B
αV8-20
αV8-18
αV8-12
αV8-10
αV8-4
- - - - + + PCP
- + - + - + EndoH
Coomassie
Fluorogram

Figure 2-5. Proteolytic mapping of sites of [^{3}H]ethidium diazide incorporation into the nAChR α-subunit using *S. aureus* V8 protease.

nAChR-rich membranes (800 μg) were labeled with [^{3}H]ethidium diazide in the presence of 10 mM oxidized glutathione and 2 mM carbamylcholine and the absence (lanes 1 & 2) or presence (lanes 3 & 4) of PCP and split for incubation with (lanes 2 & 4) or without (lanes 1 & 3) EndoglycosidaseH as described in methods. After incubation membranes were submitted to SDS-PAGE, and the α-subunit was excised. The excised bands were transferred to the well of a 15% mapping gel and digested with V8 protease as described in methods. A, mapping gel stained with Coomassie Blue. B, fluorogram of mapping gel, exposed for 6 weeks. The mobility of the proteolytic fragments is indicated on the left. The incorporation in each polypeptide was additionally measured by scintillation counting of excised gel pieces. Incorporation in the presence of carbamylcholine (+/-) or the presence of carbamylcholine and PCP (+/+) in each α-subunit fragment: αV8-20: +/-: 2271 cpm, +/+: 832 cpm; αV8-18: +/-: 390 cpm, +/+: 181 cpm; αV8-10 (including αV8-12): +/-: 346 cpm, +/+: 251 cpm (αV8-12: +/-: 262 cpm; +/+: 185 cpm); αV8-4: +/-: 43 cpm, +/+: 26 cpm. +EndoH: αV8-20: +/-: 1618 cpm, +/+: 533 cpm; αV8-18: +/-: 126 cpm, +/+: 60 cpm; αV8-10 (including αV8-12): +/-: 38 cpm, +/+: 45 cpm (αV8-12: +/-: 282 cpm, +/+: 210 cpm); αV8-4: +/-: 27 cpm, +/+: 23 cpm. Shown above is a schematic indicating the positions of the four fragments within the primary structure of the nAChR α subunit.