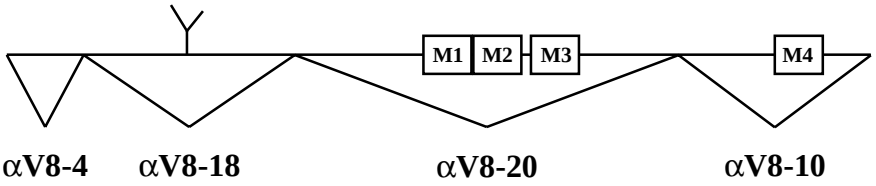
M1
M2
M3
M4
αV8-4
αV8-18
αV8-20
αV8-10

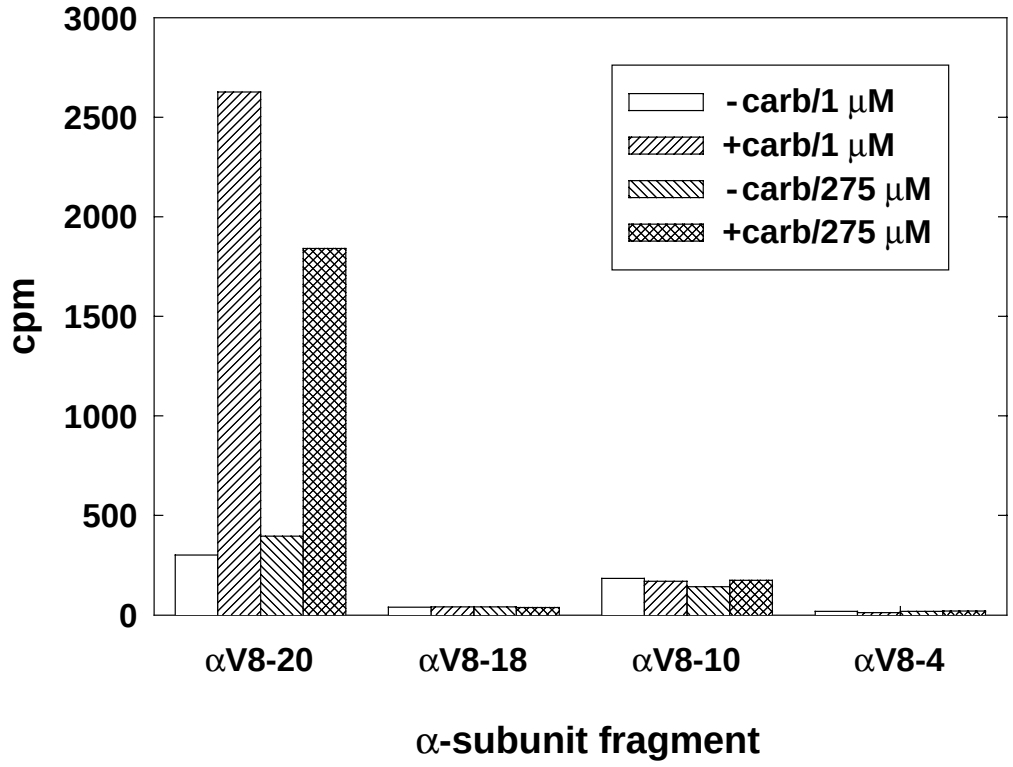
3000
2500
2000
1500
1000
500
0
cpm
-carb/1 μM
+carb/1 μM
-carb/275 μM
+carb/275 μM
αV8-20
αV8-18
αV8-10
αV8-4
α-subunit fragment

Figure 3-7. Proteolytic mapping of sites of [^{3}H]3-azioctanol incorporation into the nAChR α-subunit using *S. aureus* V8 protease.

nAChR-rich membranes (400 μg at 2 mg/ml) were labeled with 1 μM (11 Ci/mmol) or 275 μM (0.04 Ci/mmol) [^{3}H]3-azioctanol in the absence or presence of 2 mM carbamylcholine. After photolysis at 365 nm for 10 minutes, membranes were pelleted, resuspended in sample buffer, and submitted to SDS-PAGE. Following electrophoresis, the α-subunit was excised and transferred to the well of a 15% mapping gel for digestion with V8 protease. Bands were visualized with Coomassie blue, and ^{3}H incorporation was quantified by scintillation counting. ^{3}H present in proteolytic fragments of nAChR α-subunit labeled in the absence or presence of 2 mM carbamylcholine at 1 μM and 275 μM [^{3}H]3-azioctanol is shown. A schematic of digestion of α-subunit with V8 protease is shown above.