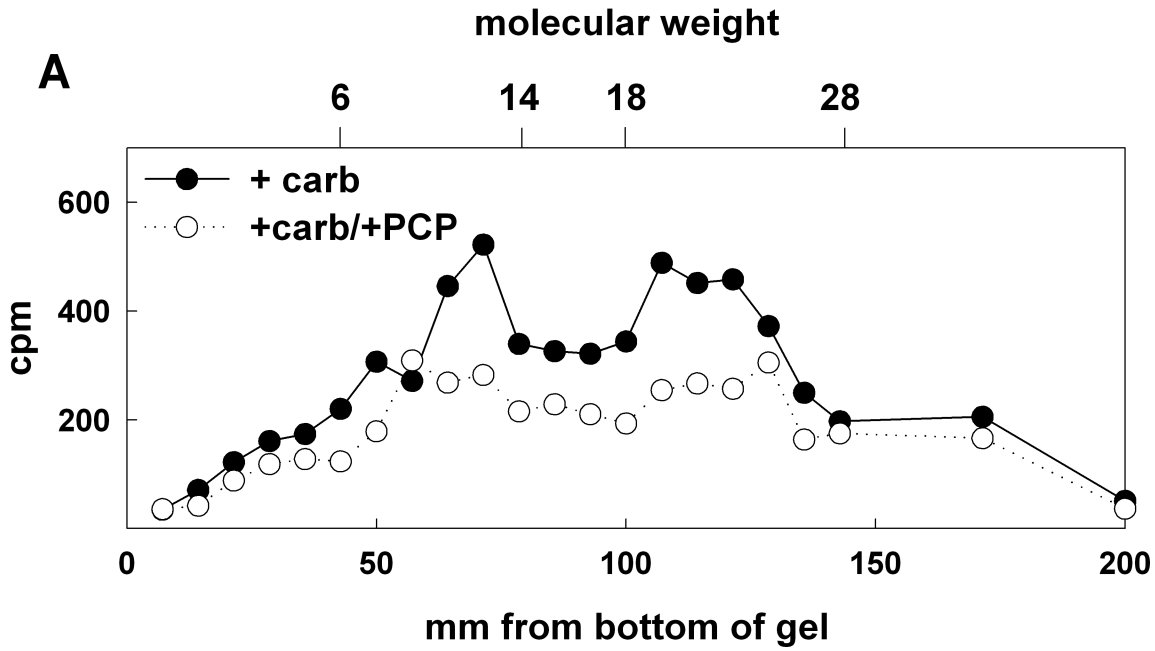
A
molecular weight
6
14
18
28
+ carb
+carb/+PCP
600
400
200
cpm
0
50
100
150
200
mm from bottom of gel

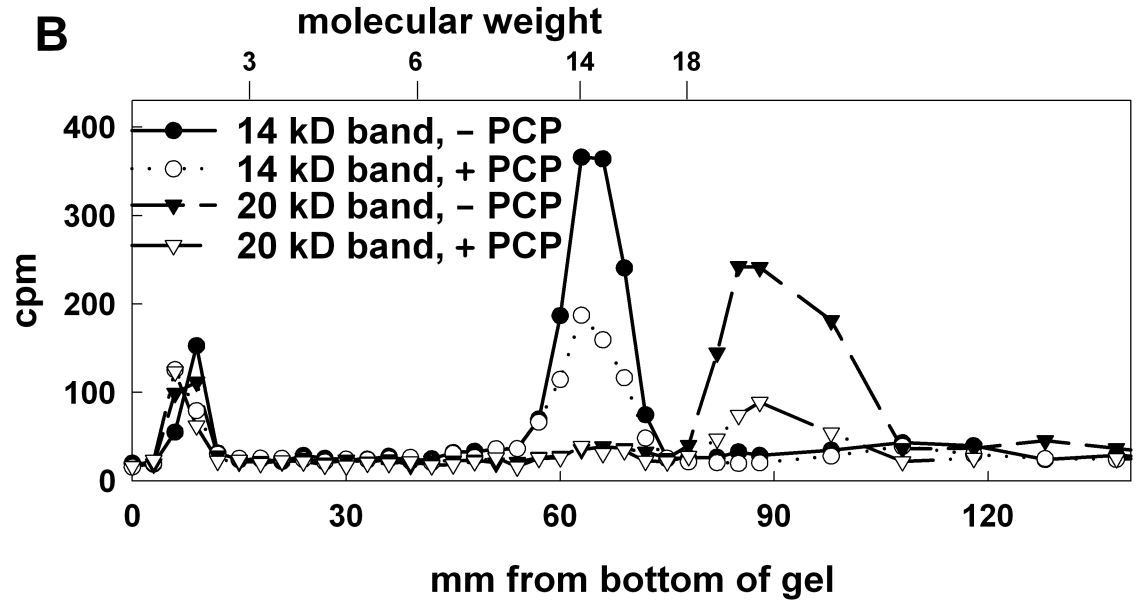
B
molecular weight
3
6
14
18
14 kD band, – PCP
14 kD band, + PCP
20 kD band, – PCP
20 kD band, + PCP
400
300
200
100
0
cpm
0
30
60
90
120
mm from bottom of gel

Figure 2-8. Proteolytic mapping of the sites of [^{3}H]ethidium diazide incorporation in the nAChR δ-subunit using V8 protease.

A. nAChR-rich membranes (400 μg) were equilibrated with [^{3}H]ethidium diazide in TPS in the presence of carbamylcholine and the absence (●) or presence of PCP (○). After photolysis in the presence of 10 mM GSSG, the samples were subjected to SDS-PAGE, and the δ-subunit was excised after brief staining with Coomassie Blue. The excised bands were transferred to the wells of a 15% mapping gel and digested with V8 protease. The lanes were cut into 5 mm slices for the lower 100 mm and 20 mm slices for the upper portion of the gel. ^{3}H was quantified by scintillation counting. The mobility of molecular weight markers is indicated along the top axis.

B. Aliquots of ~14 kD (δV8-14, ●, ○) and ~20 kD (δV8-20, ▼, ▽) bands isolated from V8 mapping gel of nAChR δ-subunit labeled in the absence (●, ▼) and presence (○, ▽) of PCP were diluted in sample buffer and submitted to SDS-PAGE using a 15% mapping gel without V8 protease. Gel was run at constant current overnight until dye front reached the bottom of the gel. Lanes were cut into 2 mm slices, and ^{3}H was quantified by scintillation counting. The mobility of molecular weight markers is indicated along the top axis.