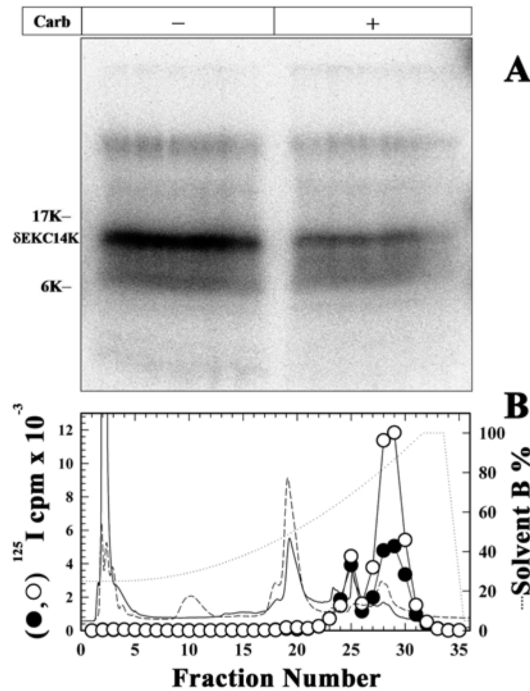
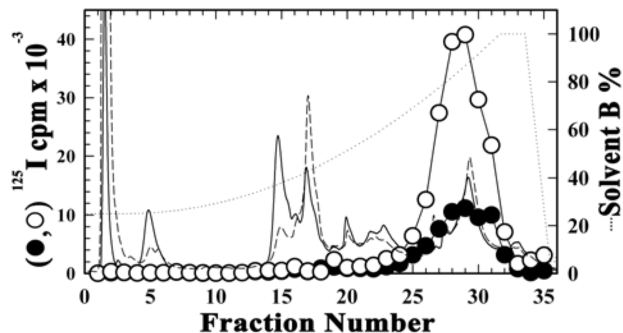


Supplemental Figure S1. Isolation of [^{125}I]TID-labeled δM1 and δM2 by Tricine SDS-PAGE and reversed phase-HPLC fractionation of EndoLys-C digests of δ subunits. Delta subunits were isolated from purified nAChRs photolabeled with [^{125}I]TID in the absence (–Carb, ○) or presence of agonist (+Carb, ●). **A**, a phosphor image of the Tricine gel fractionation of the EndoLys-C digests of the δ subunits. **B**, the major radioactive bands, which run with an apparent molecular mass of 14 kDa, were excised, and the peptides recovered from those bands were fractionated by reversed-phase HPLC, as described under "Experimental Procedures". The elution of peptides was monitored by the absorbance at 215 nm (solid lines) and fluorescence (dashed line), and the ^{125}I in each fraction was determined by γ-counting (○, ●). Sequence analysis of the pools of fractions 27-30, which contained a fragment beginning at the N-terminus of δM2, are shown in Figures 1C and 1D. Sequence analysis of the pools of fractions 23-25 (Figure 2C) identified a fragment beginning at δPhe206 that extended through δM1.



Supplemental Figure S2. Reversed-phase HPLC fractionation of V8 protease digests of δ subunits isolated from purified nAChRs photolabeled with [^{125}I]TID in the absence (−Carb, ○) or presence of agonist (+Carb, ●). The elution of peptides was monitored by the absorbance at 215 nm (solid lines) and fluorescence (dashed line), and the ^{125}I in each fraction was determined by γ-counting (○, ●). To identify labeling in the δM2-M3 loop HPLC fractions were pooled for sequencing (Figure 2D) with the samples treated with OPA at sequencing cycle 6 to prevent further sequencing of any peptide not containing a proline in cycle 6 of Edman degradation.