



Figure 3-10. Reverse phase HPLC purification of [³H]3-azidoctanol labeled α V8-18 and sequence analysis of HPLC fraction.

A. α V8-18 isolated from nAChR labeled with 275 μ M [³H]3-azidoctanol in the absence (●) or presence of 10 μ M α BgTx (▼) or 2 mM carbamylcholine (○) was applied to a Brownlee Aquapore C4 column and purified by reverse-phase HPLC. Upper panel, ³H elution profiles (5% of each fraction). Lower panel, fluorescence (·····) and absorbance profiles (—).

B. ³H (●, ○, ▼) and mass released (▲, △) on N-terminal sequencing of material from HPLC fraction 23. The sample labeled in the absence (●, ▲, △) or presence of α BgTx (▼) or carbamylcholine (○) showed two sequences, one beginning at α Val-46 and one beginning at α Thr-52 (–carb: α Val-46 (△) I_0 =41 pmol, R=92%, α Thr-52 (▲) I_0 =38 pmol, R=94%, 10120 cpm loaded, 2400 cpm remaining after 15 cycles; + α BgTx: α Val-46 I_0 =19 pmol, R=91%, α Thr-52 I_0 =24 pmol, R=91%, 10320 cpm loaded, 2000 cpm remaining after 8 cycles; +carb: α Val-46 I_0 =32 pmol, R=92%, α Thr-52 I_0 =31 pmol, R=94%, 9800 cpm loaded, 1800 cpm remaining after 15 cycles). The two sequences that were present are shown along top axis.