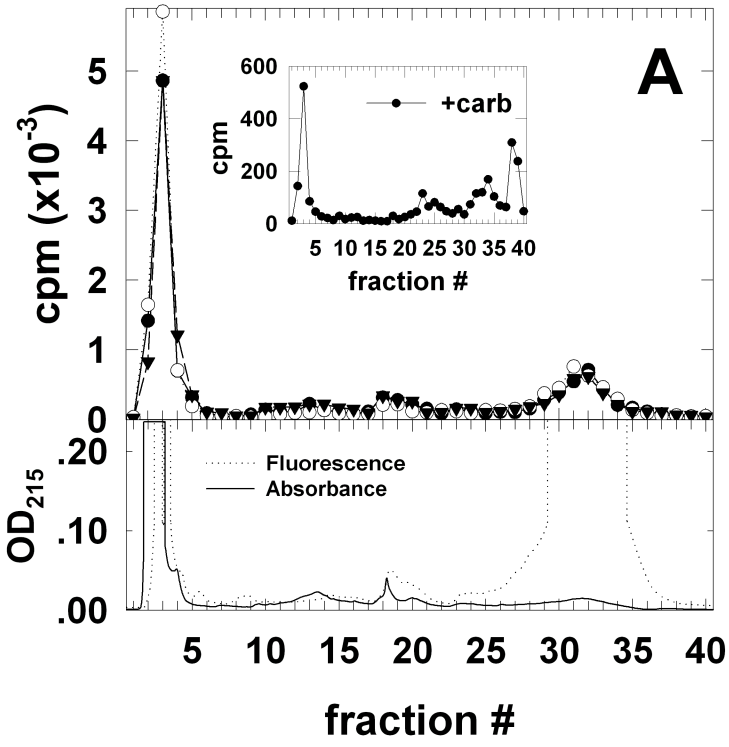
A
cpm (x10-3)
5
4
3
2
1
0
600
400
200
0
cpm
+carb
5 10 15 20 25 30 35 40
fraction #
OD215
.20
.10
.00
Fluorescence
Absorbance
5 10 15 20 25 30 35 40
fraction #

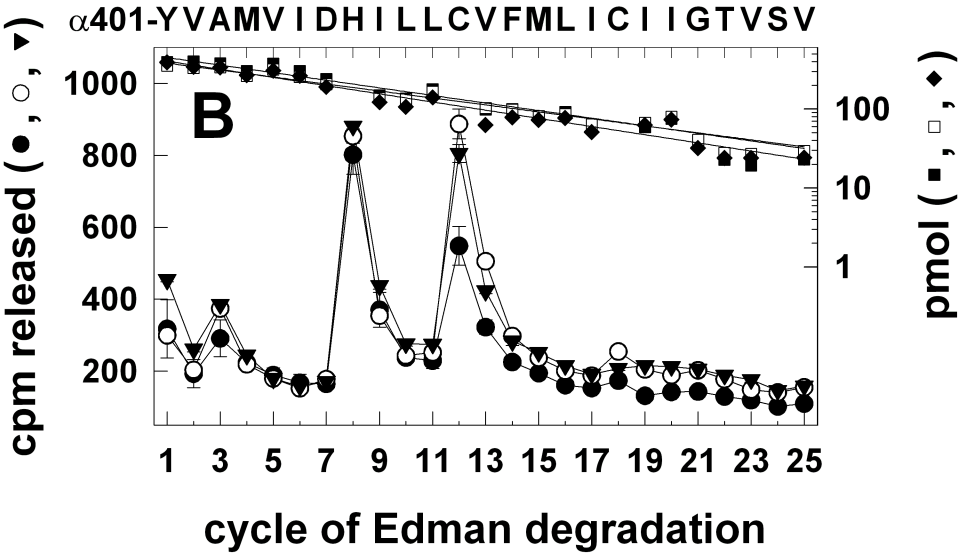
α401-YVAMV I DH I LLCVFMLICI IGTVSV
B
cpm released (●, ○, ▼)
1000
800
600
400
200
100
10
1
pmol (■, □, ◆)
1 3 5 7 9 11 13 15 17 19 21 23 25
cycle of Edman degradation

Figure 3-11. Reverse phase HPLC purification of [^{3}H]3-azioctanol labeled fragments from trypsin digestion of αV8-10 and sequence analysis of HPLC fractions.

A. αV8-10 labeled with 275 μM [^{3}H]3-azioctanol in the absence (●) or presence of 10 μM αBgTx (▼) or 2 mM carbamylcholine (○) was digested with trypsin and applied to a Brownlee Aquapore C4 column for fractionation by reverse-phase HPLC. Upper panel, ^{3}H elution profiles from the digest of αV8-10 labeled under the given conditions. Lower panel, fluorescence (·····) and absorbance profiles (—). Inset, ^{3}H elution profile of undigested αV8-10 labeled with 1 μM [^{3}H]3-azioctanol in the presence of 2 mM carbamylcholine purified by reverse-phase HPLC.

B. ^{3}H (●, ○, ▼) and mass released (■, □, ◆) on N-terminal sequencing of material from HPLC fractions 31-34. The sample labeled in the absence (●, ■) or presence of αBgTx (▼, ◆) or carbamylcholine (○, □) showed a primary sequence beginning at αTyr-401 and a secondary sequence beginning at αSer-388 (–carb: αTyr-401 I_0=502 pmol, R=90%, αSer-388 I_0=68 pmol, R=87%, 52400 cpm loaded, 12700 cpm remaining after 25 cycles; +αBgTx: αTyr-401=I_0 457 pmol, R=89%, αSer-388 I_0=70 pmol, R=87%, 48500 cpm loaded, 16700 cpm remaining after 25 cycles; +carb: αTyr-401 I_0=423 pmol, R=90%, αSer-388 I_0=72 pmol, R=88%, 57800 cpm loaded, 19400 cpm remaining after 25 cycles). The primary sequence is shown along top axis.