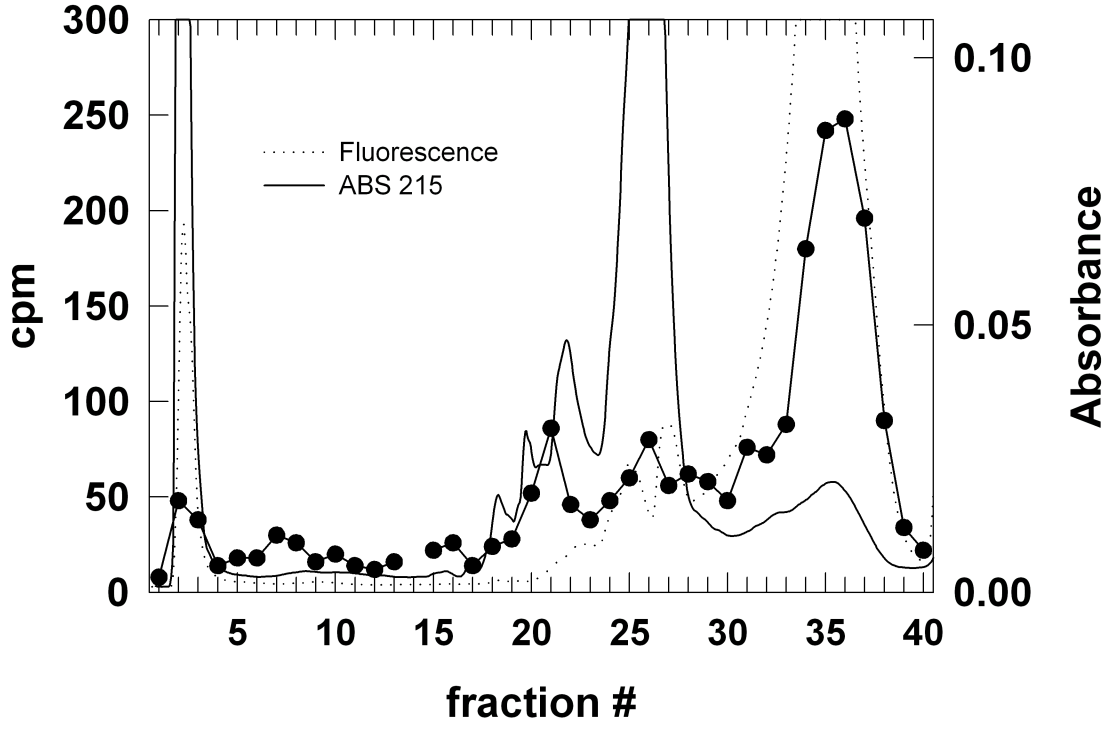
300
250
200
150
100
50
0
cpm
0.10
0.05
0.00
Absorbance
Fluorescence
ABS 215
5
10
15
20
25
30
35
40
fraction #

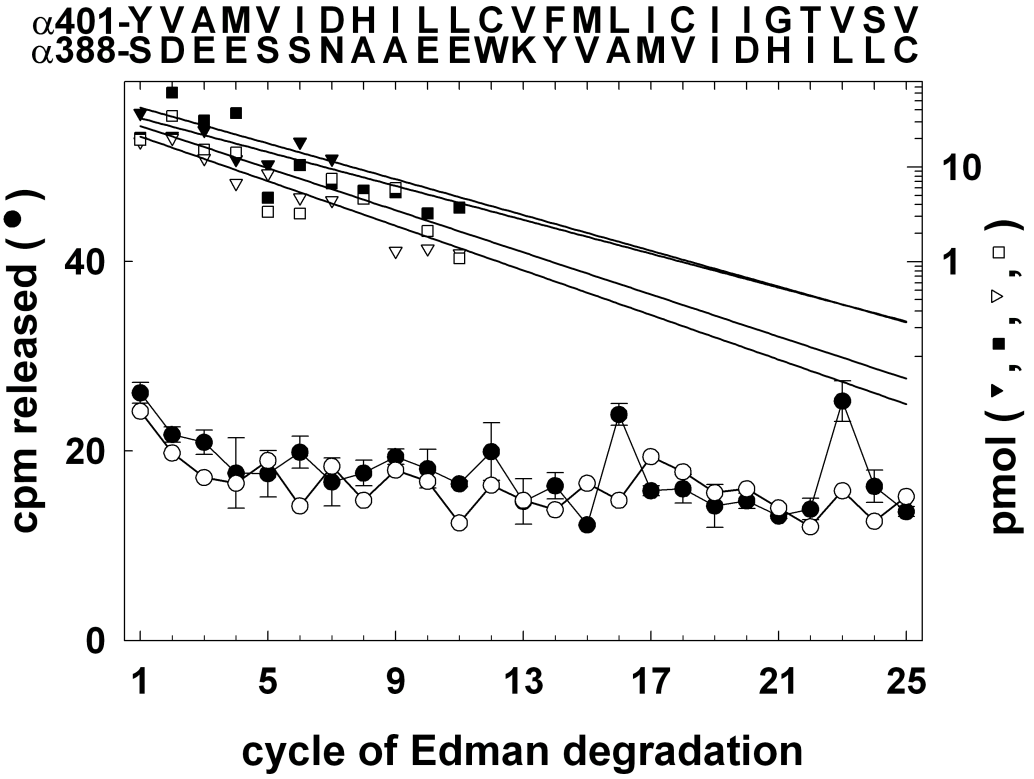
α401-Y V A M V I D H I L L C V F M L I C I I G T V S V
α388-S D E E S S N A A E E W K Y V A M V I D H I L L C
40
20
0
cpm released (●)
10
1
pmol (▼ , ■ , ▽ , □)
1
5
9
13
17
21
25
cycle of Edman degradation

Figure 4-4. Reverse phase HPLC purification of [^{3}H]progestin aryl azide labeled fragments from trypsin digest of αV8-10

A, αV8-10 isolated from nAChR photolabeled with [^{3}H]progestin aryl azide was digested with trypsin. The digest was applied to a Brownlee Aquapore C4 column and fractionated by reverse-phase HPLC. ^{3}H elution profiles (●) are from scintillation counting of 10% of each fraction. Fluorescence (·····) and absorbance profiles (—) were measured as described in methods.

B, ^{3}H (●,○) and mass released (■, ▼, □, ▽) on N-terminal sequencing of material in HPLC fractions 34-36 from two separate preparative labeling experiments. In one experiment (●), two sequences containing αM4 were present, one beginning at αTyr-401 (■, I_o=27 pmol, R=83%), and one beginning at αSer-388 (▼, I_o=25 pmol, R=83%), as well as a sequence beginning at αMet-243 (I_o=10 pmol, R=86%), containing the αM2 and αM3 segments. Total ^{3}H in the HPLC fractions was 6000 cpm, with 1300 cpm released during pre-wash, and 1300 cpm remaining on the filter. In the other experiment (○), only the sequences containing αM4 were present (αTyr-401 (□): I_o=15 pmol, R=80%; αSer-388 (▽): I_o=20 pmol, R=82%). Total ^{3}H in the HPLC fractions was 3500 cpm, with 1100 cpm released during pre-wash, and 310 cpm remaining on the filter.