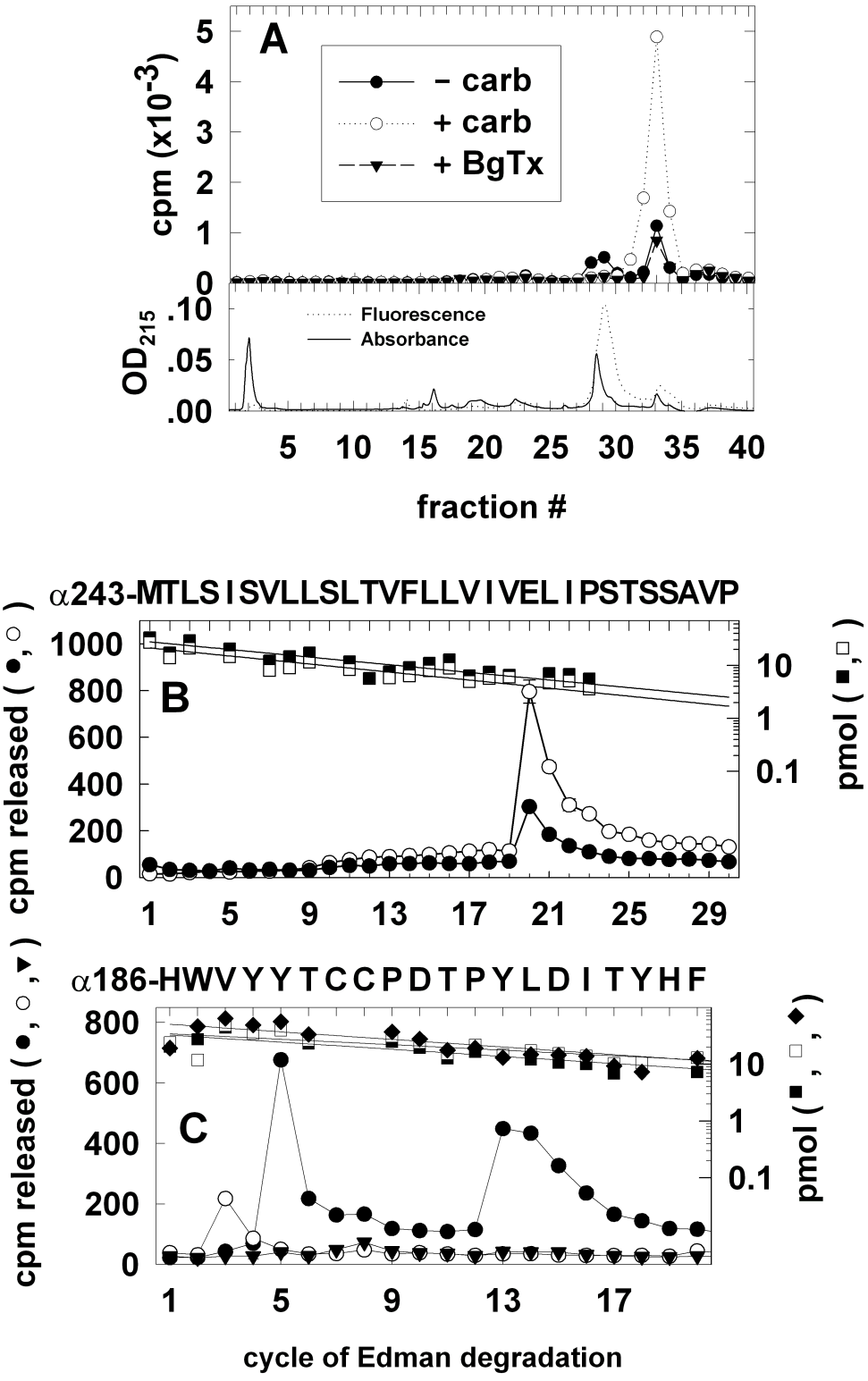
A
cpm (x10-3)
5
4
3
2
1
0
– carb
+ carb
+ BgTx
OD215
.10
.05
.00
Fluorescence
Absorbance
5 10 15 20 25 30 35 40
fraction #
α243-MTLSISVLLSLTVFLLVIVELIPSTSSAVP
cpm released (●, ○)
1000
800
600
400
200
0
B
10
1
0.1
pmol (■, □)
1 5 9 13 17 21 25 29
α186-HWVYYTCCPDTPYLDITYHF
cpm released (●, ○ ,▼)
800
600
400
200
0
C
10
1
0.1
pmol (■, , □, ◆)
1 5 9 13 17
cycle of Edman degradation

Figure 3-8. Reverse phase HPLC purification of [^{3}H]3-azioctanol labeled fragments from an EndoLysC digest of αV8-20 and sequence analysis of HPLC fractions

A. αV8-20 isolated from nAChRs photolabeled with 275 μM [^{3}H]3-azioctanol in the absence (●) or presence of 10 μM αBgTx (▼) or 2 mM carbamylcholine (○) was digested with EndoLysC. The digest was applied to a Brownlee Aquapore C4 column and fractionated by reverse-phase HPLC. Upper panel, ^{3}H elution profiles (5% of each fraction counted). Lower panel, fluorescence (·····) and absorbance profiles (—).
B, C. ^{3}H (●, ○, ▼) and mass released (■, □, ◆) on N-terminal sequencing of material in HPLC fraction 33 (B) and 29 (C). B, Fraction 33 from the sample labeled in the absence (●, ■) and presence of carbamylcholine (○, □) showed a single sequence, beginning at αMet-243, the N-terminus of the αM2 segment (–carb: I_0=23 pmol, R=92%, 9800 cpm loaded, 3900 cpm remaining after 30 cycles; +carb: I_0=30 pmol, R=92%, 26000 cpm loaded, 3900 cpm remaining after 30 cycles). C, Fraction 29 from the sample labeled in the absence (●, ■) or presence of αBgTx (▼, ◆) or carbamylcholine (○, □) showed a primary sequence beginning at αHis-186 and a secondary sequence beginning at αAsp-180 (–carb: αHis-186 I_0=35 pmol, R=93%, αAsp-180 I_0=4.6 pmol, R=86%, 16700 cpm loaded, 3400 cpm remaining after 25 cycles; +αBgTx: αHis-186 I_0=55 pmol, R=93%, αAsp-180 I_0=2.4 pmol, R=95%, 4100 cpm loaded, 1000 cpm remaining after 25 cycles; +carb: αHis-186 I_0=36 pmol, R=95%, αAsp-180 I_0=7.8 pmol, R=82%, 4800 cpm loaded, 1200 cpm remaining after 25 cycles). Primary sequence for each fraction is shown on top axes.