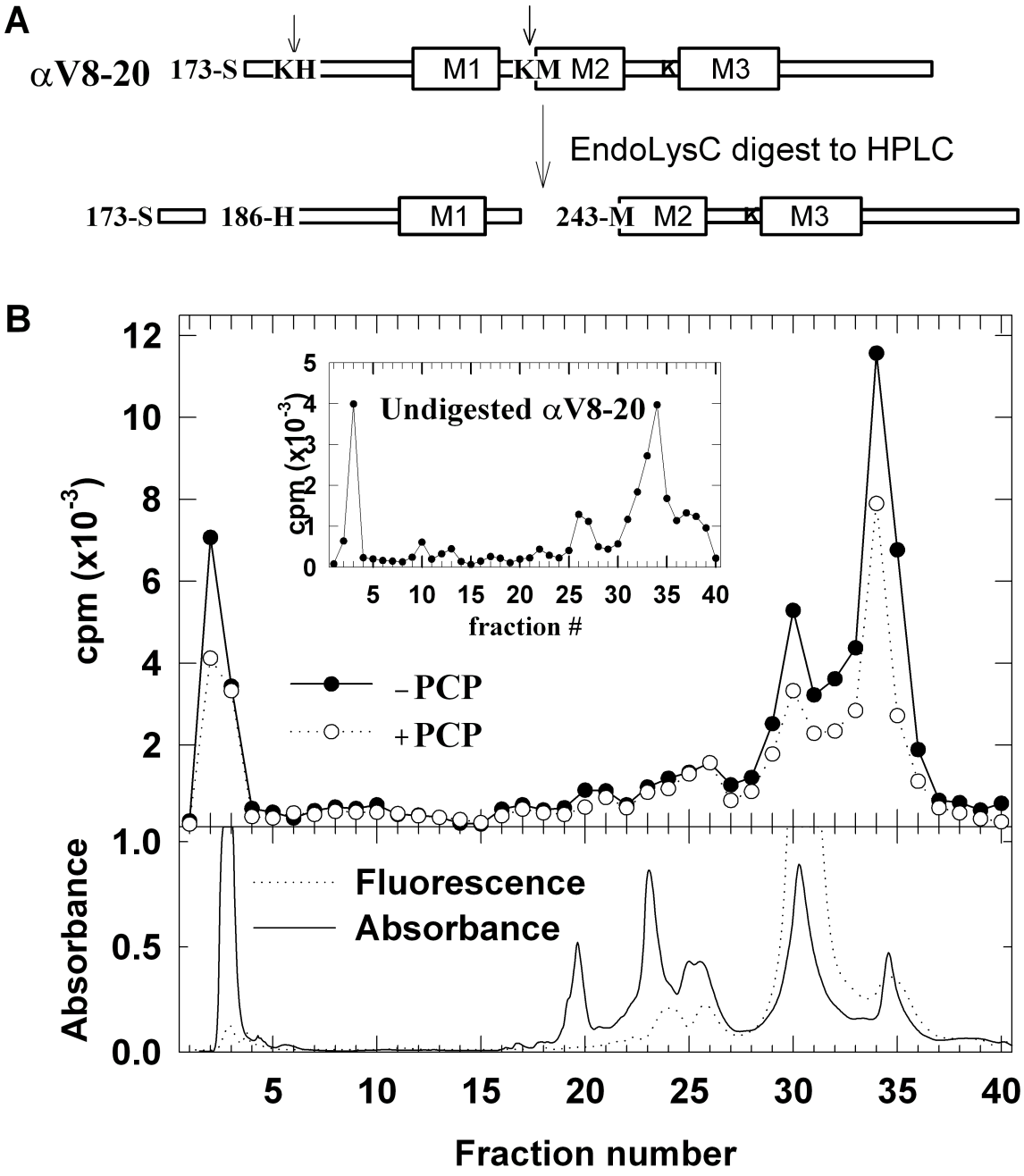
A
αV8-20
173-S
KH
M1
KM
M2
M3
EndoLysC digest to HPLC
173-S
186-H
M1
243-M
M2
M3
B
cpm (x10-3)
Undigested αV8-20
cpm (x10-3)
fraction #
– PCP
+ PCP
Absorbance
Fluorescence
Absorbance
Fraction number

Figure 2-6. Reverse-phase HPLC purification of [^{3}H]ethidium diazide labeled fragments from EndoLysC digest of αV8-20.

A. A map of the amino acid sequence of the nAChR α-subunit contained within αV8-20. Rectangles, M1, M2 and M3 hydrophobic segments. Arrows, location of two known sites of EndoLysC cleavage. The resulting cleavage products are indicated. Other potential cleavage sites indicated by K.

B. EndoLysC digested αV8-20 isolated from nAChR-rich membranes photolabeled with [^{3}H]ethidium diazide in the presence of carbamylcholine and the absence (●) or presence of PCP (○) 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 profile calculated from a 10% aliquot of each fraction. Lower panel, fluorescence (·····) and absorbance profiles (—). Inset, ^{3}H elution profile when αV8-20 (+/-) isolated by SDS-PAGE was purified by reverse-phase HPLC.