



**Figure 2-7.  $^3\text{H}$  and mass release upon N-terminal sequence analysis of HPLC fractions of EndoLysC-digest of [ $^3\text{H}$ ]ethidium diazide labeled  $\alpha\text{V8-20}$  .**

A. Sequence analysis of fraction 34 from HPLC purification of Figure 2-6 (upper panel) and the same fraction from the purification of a second, independent labeling experiment. For each sample, 67% of each cycle of Edman degradation was analyzed for released  $^3\text{H}$  ( $\bullet, \circ$ ), and 33% for released PTH-amino acids ( $\blacksquare, \square$ ). For both labeling conditions, the only sequence detected began at  $\alpha\text{Met-243}$ , the N-terminus of  $\alpha\text{M2}$ . Labeling 1 (upper panel): +/- ( $\bullet, \blacksquare$ ):  $I_0=109$  pmol,  $R=90\%$ , 11600 cpm loaded, 2600 cpm remaining after 25 cycles. ++ ( $\circ, \square$ ):  $I_0=159$  pmol,  $R=88\%$ , 7900 cpm loaded, 1800 cpm remaining after 25 cycles. Labeling 2 (lower panel): +/- ( $\bullet, \blacksquare$ ):  $I_0=98$  pmol,  $R=90\%$ , 17000 cpm loaded, 5300 cpm remaining after 25 cycles. ++ ( $\circ, \square$ ):  $I_0=63$  pmol,  $R=87\%$ , 7000 cpm loaded, 2500 cpm remaining after 25 cycles. The sequence of the identified peptide is shown above.

B. Sequence analysis of fraction 29 from HPLC purification of Figure 2-6. As above, for each sample, 67% of each cycle of Edman degradation was analyzed for released  $^3\text{H}$  ( $\bullet, \circ$ ), and 33% for released PTH-amino acids ( $\blacksquare, \square$ ). For both labeling conditions, the primary sequence detected began at  $\alpha\text{His-186}$  and a secondary sequence beginning at  $\alpha\text{Asp-180}$  (+/- ( $\bullet, \blacksquare$ ):  $\alpha\text{His-186}$   $I_0=128$  pmol,  $R=92\%$ ;  $\alpha\text{Asp-180}$   $I_0=18$  pmol,  $R=94\%$ , 5290 cpm loaded, 1250 cpm remaining after 25 cycles. ++ ( $\circ, \square$ ):  $\alpha\text{His-186}$   $I_0=208$  pmol,  $R=91\%$ ,  $\alpha\text{Asp-180}$   $I_0=39$  pmol,  $R=91\%$ , 3330 cpm loaded, 770 cpm remaining after 25 cycles). Level of released PTH-amino acids of the primary sequence is plotted ( $\blacksquare, \square$ ), and the sequence of the primary peptide is shown above.