

Figure 4-6. HPLC purification of [3 H]progestin aryl azide labeled fragments from trypsin digest of γ -subunit

A, B. nAChR γ-subunit isolated from nAChR-rich membranes photolabeled with [³H]progestin aryl azide was digested with trypsin. The digest was initially fractionated by Tricine SDS-PAGE. Two bands containing ³H (Band 2, 3-6 kD (A); Band 1, 1-3 kD (B)) were applied to a Brownlee Aquapore C4 column and fractionated by reverse-phase HPLC. ³H elution profiles (•) are from scintillation counting of 10% of each fraction. Fluorescence (****) and absorbance profiles (—) were measured as described in methods. For Band 2 (A), fraction 31 was sequenced, and for Band 1 (B) fractions 25 and 26 were sequenced (see Table 4-1).