Abstract
The mitochondrial porin or VDAC (Voltage-Dependent Anion Channel), the pore-forming structure responsible for the high permeability of the outer mitochondrial membrane, was found to be one of only three mitochondrial proteins bound by [14C]dicyclohexylcarbodiimide (DCCD) at low dosages (1.5 nmol/mg of mitochondrial porin) (De Pinto, V., Tommasino, M., Benz, R., and Palmieri, F. (1985) Biochim. Biophys. Acta 813, 230-242). Treatment of intact mitochondria with DCCD results in the inhibition of their ability to binding hexokinase (Nakashima, R. A., Mangan, P. S., Colombini, M., and Pedersen, P. L. (1986) Biochemistry 25, 1015-1021). In the present study, mitochondrial porin was purified from [14C]DCCD-labeled mitochondria. The purified labeled porin was treated with the cleavage reagent CNBr and with the endoproteases trypsin and V8 from Staphylococcus aureus and blotted to polyvinylidene difluoride membrane. The transferred peptides were detected with Coomassie Blue dye, excised, and sequenced. The sequences of several labeled and unlabeled peptides were obtained and then overlapped. The region containing the [14C]DCCD radioactivity was limited to 50 amino acid residues and completely sequenced. Covalently incorporated [14C]DCCD was exclusively released at the position corresponding to glutamate 72. The DCCD-reactive residue is located in the 4th of 16 predicted transmembrane amphipathic beta-strands. When the sequence surrounding the DCCD site was compared to those surrounding the DCCD-reactive residue of other membrane proteins, no homology was apparent.