Positive residues involved in the voltage-gating of the mitochondrial porin-channel are localized in the external moiety of the pore
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Abstract
The role of positive charges located on the hydrophilic surface of the mitochondrial outer membrane channel was investigated by studying the interaction between LDAO-solubilized porin and a cation-exchanger column. The binding of porin to the column material was inhibited when the elution buffer had a pH of 9 or when 2 mM dextran sulfate was added to the buffer at neutral pH. Interestingly, the addition of a synthetic copolymer of methacrylate, maleate and styrene known as a potent modulator of the voltage-dependence, did not influence the interaction between column material and porin. Incubation of porin with fluorescein isothiocyanate (FITC) resulted in the isolation of a porin fraction in which on average two lysines located on the surface of the pore-forming complex per 35 kDa polypeptide were modified. The voltage-dependence of the fluorescein isothiocyanate modified porin was strongly decreased as compared with the unmodified porin. The experiments presented here give the first biochemical evidence that positively charged lysine residues located on the surface of the channel-forming complex are responsible for the gating of the mitochondrial porin-channel.