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The conformation of human phospholipid scramblase 1, as studied by infrared spectroscopy. Effects of calcium and detergent.

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ABSTRACT

Human phospholipid scramblase 1 (SCR) is a membrane protein that catalyzes the transmembrane (flip-flop) motion of phospholipids. It can also exist in a non membrane-bound form in the nucleus, where it modulates several aspects of gene expression. Catalysis of phospholipid flip-flop requires the presence of millimolar Ca^{2+}, and occurs in the absence of ATP. Membrane-bound SCR contains a C-terminal α-helical domain embedded in the membrane bilayer. The latter domain can be removed giving rise to a stable truncated mutant SCRΔ that is devoid of scramblase activity. In order to improve our understanding of SCR structure infrared spectra have been recorded of both the native and truncated forms, and the effects of adding Ca^{2+}, or removing detergent, or thermally denaturing the protein have been observed. Under all conditions the main structural component of SCR/SCRΔ is a β-sheet. Removing the C-terminal 28 aa residues, which anchor SCR to the membrane, leads to a change in tertiary structure and an increased structural flexibility. The main effect of Ca^{2+} is an increase in the α/β ratio of secondary structure components, with a concomitant increase in the proportion of non-periodic structures. At least in SCRΔ, detergent (Zwittergent 3-12) decreases the structural flexibility, an effect somewhat opposite to that of increasing temperature. Thermal denaturation is affected by Ca^{2+}, detergent, and by the presence or absence of the C-terminal domain, each of them influencing in different ways the denaturation pattern.

Keywords: Scramblase, membranes, calcium, protein structure, detergents, infrared spectroscopy, 2D IR correlation spectroscopy

1. INTRODUCTION

Phospolipid scramblases catalyze the transbilayer (flip-flop) motion of phospholipid molecules in an ATP-independent process. The first member of this family, human phospholipid scramblase 1 (from now on abbreviated as SCR), was described in 1996 (1). This was soon followed by the discovery of three other members of the same family (2). The early results in the field were reviewed by Sahu et al. (3). More recently Suzuki et al. (4) described phospholipid scrambling induced by a transmembrane protein (TMEM16F) in animal cells that has been tentatively associated to the Scott syndrome. Phospholipid scramblases usually require the presence of Ca^{2+} for their catalytic action.

SCR is a 318aa, type II endofacial membrane protein, expressed in a variety of cells. It contains a proline-rich domain at the N-terminus, a DNA-binding region (aa 86-118), a palmitoylation motif (aa 184-189), a nuclear localization signal (257-266), a Ca^{2+}-binding site (aa 273-284), and a transmembrane α-helix (aa 291-309) (Scheme 1). The Ca^{2+}- binding motif has a Ca^{2+} affinity constant in the millimolar range,