

Cloning, expression, purification, crystallization and X-ray crystallographic analysis of recombinant human C1ORF123 protein

ABSTRACT

C1ORF123 is a human hypothetical protein found in open reading frame 123 of chromosome 1. The protein belongs to the DUF866 protein family comprising eukaryote-conserved proteins with unknown function. Recent proteomic and bioinformatic analyses identified the presence of C1ORF123 in brain, frontal cortex and synapses, as well as its involvement in endocrine function and polycystic ovary syndrome (PCOS), indicating the importance of its biological role. In order to provide a better understanding of the biological function of the human C1ORF123 protein, the characterization and analysis of recombinant C1ORF123 (rC1ORF123), including overexpression and purification, verification by mass spectrometry and a Western blot using anti-C1ORF123 antibodies, crystallization and X-ray diffraction analysis of the protein crystals, are reported here. The rC1ORF123 protein was crystallized by the hanging-drop vapor-diffusion method with a reservoir solution comprised of 20% PEG 3350, 0.2 M magnesium chloride hexahydrate, 0.1 M sodium citrate pH 6.5. The crystals diffracted to 1.9 Å resolution and belonged to an orthorhombic space group with unit-cell parameters $a = 59.32$, $b = 65.35$, $c = 95.05$ Å. The calculated Matthews coefficient (VM) value of $2.27 \text{ \AA}^3 \text{ Da}^{-1}$ suggests that there are two molecules per asymmetric unit, with an estimated solvent content of 45.7%.

Keyword: C1ORF123; Hypothetical protein; DUF866; Polycystic ovary syndrome; Bioinformatic analysis