ASSEMBLED RECOMBINANT CHLOROPLAST C-RING IN *ESCHERICHIA COLI*

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ATP synthase is a protein that provides cells with adenosine triphosphate (ATP), which is the main source of energy for the majority of biochemical processes in living organisms. ATP synthase converts transmembrane electrochemical potential into mechanical rotation of the c-ring rotor in the membrane part (F_o) and the central stalk (γ/ε) of the protein complex, triggering corresponding conformational changes in ATP catalytic centers [1].

The key element of ATP synthase is its membrane rotor domain c-ring, consisting of n protomers (c_1 subunits). The number of protons requiring to pass through the c-ring for the synthesis of a single ATP molecule is determined by the stoichiometry of the c-ring. The stoichiometry is constant within each specie, but can significantly vary between distant lineages. Target mutations allow to change c-ring stoichiometry and are described in literature [2]. However, the role of the surrounding environment is not sufficiently studied yet. For instance, it is not proven that c-rings with identical amino acid sequences, assembled in different species, will have the same stoichiometry.

In this work, a recombinant c-ring from ATP synthase from spinach chloroplasts, expressed in *Escherichia coli*, was studied. Optimal expression parameters for providing assembly of the complex were selected. It was shown that polyhistidine tag (His-tag) interferes with assembly, thus a genetic construction without His-tag was used. The protein was isolated and purified via the following biochemical methods: centrifugation, precipitation at the step of ammonium sulfate, and anion-exchange chromatography. The sample of purified c-ring was then biochemically and structurally characterized and shown to most likely consist of 14 subunits, which corresponds with the stoichiometry of the native chloroplast c-ring. It is notable that the completed c-ring was located exclusively in the water-soluble fraction of the cell lysate, while the membrane hydrophobic fraction mostly contained c_1 subunits. The acquired sample was studied by electron microscopy with negative contrast.

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