Biochemical and biophysical characterization of a truncated NADH dehydrogenase-2 of *Escherichia coli*

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NADH dehydrogenase-2 (NDH-2) of *Escherichia coli* is a flavoprotein bound to the membrane by its C-terminal region. We have constructed a water soluble protein, Trun-3, eliminating the last 43 aminoacids of NDH-2. Despite FAD cofactor was absent in the purified truncated protein, its enzymatic activity was reconstituted by the addition of 10 µM FAD. Here, far-UV circular dichroism (CD), Fourier transform infrared (FTIR) spectroscopy, fluorescence spectroscopy and limited proteolysis experiments provided evidence for a FAD-induced conformational change in Trun-3. For instance, a significant decrease in intrinsic fluorescence emission took place upon FAD binding, which could be related to conformational rearrangements that would affect the local environment of the protein tryptophan residues. Also, the limited digestion experiment with trypsin revealed a different fragmentation pattern in the presence of FAD compared to that of the apo-Trun-3, indicating that FAD binding has an effect on the protein conformation. However, CD data indicated that secondary structure of the apoprotein was hardly affected by the binding of the flavin since Trun-3 had the same structural component ratios independently of its cofactor. Similar results were observed by FTIR. Apparent melting temperatures and thermal inactivation kinetics showed that cofactor binding to apoenzyme lead to a slight thermal stabilization of holoenzyme. Taking together, FAD binding affects tertiary structure, while slightly changes the secondary structure of Trun-3. A broad knowledge about NDH-2s could promote their potential applications in medical science, as chemotherapeutic targets or in gene therapy.

Keywords: FAD cofactor; membrane protein; structure