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Fluorone dyes binding to extracellular and cytoplasmic domains of Na,K-ATPase

M. Havlikova[1,2], M. Huliciak[2], V. Bazgier[3], K. Berka[4] and M. Kubala[2]

[1]Department of Medical Chemistry and Biochemistry, Faculty of Medicine, Palacky University, Olomouc, Czech Republic, [2]Department of Biophysics, Faculty of Science, Palacky University, Olomouc, Czech Republic, [3]Department of Physical Chemistry, Centre of the Region Hana for Biotechnological and Agricultural Research, Faculty of Science, Palacky University, Olomouc, Czech Republic, [4]Department of Physical Chemistry, Regional Center of Advanced Technologies and Materials, Faculty of Science, Palacky University, Olomouc, Czech Republic

Combination of fluorescence techniques and molecular docking was used to monitor interaction of Na,K-ATPase and its large cytoplasmic loop connecting fourth and fifth transmembrane helices (C45) with fluorone dyes (i.e. eosin Y, 5(6)-carboxyeosin, rose bengal, fluorescein, and erythrosine B). Our data suggested that there are at least two binding sites for all used fluorone dyes, except of 5(6)-carboxyeosin. The first binding site is located on C45 loop, and it is sensitive to the presence of nucleotide. The other site is located on the extracellular part of the enzyme, and it is sensitive to the presence of Na^+ or K^+ ions. The molecular docking revealed that in the open conformation of C45 loop (which is obtained in the presence of ATP) all used fluorone dyes occupy position directly inside the ATP-binding pocket, while in the closed conformation (i.e. in the absence of any ligand) they are located only near the ATP-binding site depending on their different sizes. On the extracellular part of the protein, the molecular docking predicts two possible binding sites with similar binding energy near Asp897(α) or Gln69(β). The former was identified as a part of interaction site between α- and β-subunits, the latter is in contact with conserved FXYD sequence of the γ-subunit. Our findings provide structural explanation for numerous older studies, which were performed with fluorone dyes before the high-resolution structures were known. Further, fluorone dyes seem to be good probes for monitoring of intersubunit interactions influenced by Na^+ and K^+ binding.

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The effect of substitutions of E457 and A534 residues in thermostable mutant of *Luciola mingrelica* firefly luciferase on its activity and stability

Y. A. Modestova, M. I. Koksharov and N. N. Ugarova

Department of Chemistry, Lomonosov Moscow State University, Moscow, Russia

Firefly luciferase catalyzes firefly luciferin oxidation by O_2 in presence of ATP. It is widely applied in various bioanalytical systems and in biochemical and physiological studies. The enzyme is composed of a large N-domain (residues 1–445) and a small C-domain (residues 446–548). The roles of amino acid residues of N-domain are extensively investigated. Meanwhile information on the roles of C-domain amino acid residues is very limited.

E457 residue is highly conserved in the enzymes of ANL superfamily, including firefly luciferase. To clarify its role in firefly luciferase *L. mingrelica* we obtained mutants with following single mutations on the basis of its thermostable form 4TS: E457V, E457Q, E457D, E457K. In the molecules of pH-independent luciferases E457 residue forms two additional hydrogen bonds with R534, which are absent in 4TS and its above-mentioned mutants. To investigate the role of these bonds two mutants were obtained: in A534R mutant the H-bonds can be formed, while double E457V/A534R mutant demonstrates the effect of A534R, which is not related to the H-bond formation.

All the mutants were purified to homogeneity and extensively characterized. All mutants except E457D preserved a high level of enzymatic activity. Km(ATP) values of E457K, E457D, E457V and A534R were 2–3-folds higher than that of 4TS, Km (LH2) values were 2–3-folds lower for all the mutants except E457K. The change of sign (E457K) or total elimination (E457V) of the charge on the 457th residue lowers the enzyme thermostability by the factor of ~2. The substitution, preserving the charge on the residue (E457D), results in a smaller destabilizing effect. Introduction of additional hydrogen bonds between E457 and R534 residues (A534R) results in two-fold increase of thermostability in comparison with that of 4TS.

The contribution of low-energy red emitter in an overall bioluminescent spectrum grows with the increase of the temperature for most of the mutants, but its role is greater for the less thermostable enzymes. Meanwhile high-energy green emitter plays more significant part in the spectral characteristics of 4TS and its stable mutants (A534R and E457D). Possible explanations of the above-described effects in relation to the structural changes in luciferase molecule are proposed.

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A novel tool to shield against alpha-synuclein's toxic effects

A. Gauhar, E. Mirecka, H. Shaykhalishahi and W. Hoyer

Institute of Physical Biology, Heinrich Heine University Dusseldorf, Dusseldorf, Germany

The unstructured and aggregation-prone protein α-synuclein is linked to the pathology of a group of neurodegenerative disorders, for example Parkinson's disease, which are marked by the presence of Lewy bodies. The oligomeric and fibrillar forms of the protein are implicated as the main culprits in the process.

Here in this work the engineered binding protein Z_{syn69}, isolated from a combinatorial protein library via the phage display system, is employed to study its potential in blocking or mitigating the toxic effects of α-synuclein's fibrils and oligomers in a cell culture model. The binding protein is designed to remain functional in the cell culture milieu. Interaction of the binding protein towards α-synuclein was initially confirmed by ITC (Isothermal Titration Calorimetry), showing desirable binding affinity. α-synuclein's aggregation kinetics was analyzed by Thioflavin-T fluorescence, demonstrating thorough inhibition of aggregation at equimolar concentrations of Z_{syn69} while prolonging the lag phase of aggregation in the presence of sub-stoichiometric concentrations of Z_{syn69}. The MTT cell viability test was used to characterize the ability of Z_{syn69} in controlling the toxicity of α-synuclein fibrils in SH-SY5Y neuroblastoma cell line. Our results revealed significant reduction of toxic effect of the aggregates in the presence of the binding protein.

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Amyloid beta 1-42 oligomerization *in vitro* and characterization with SDS-PAGE, MALDI and ESI MS

M. Friedemann, V. Tougu, T. Kirsipuu and P. Palumaa

Department of Gene Technology, Tallinn University of Technology, Tallinn, Estonia

Aggregation of amyloid beta (Ab) peptides into oligomers and amyloid plaques in human brain is considered to be a causative factor in Alzheimer's disease (AD), however, the precise mecha-