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Dynamics of mitochondrial ADP/ATP carrier: Role of C-terminal region of yAAC2

Kazuto OHKURA*[1], Yasuko WATANABE[1], Yuki KAWAGUCHI[1], Yasuhiro MASUBUCHI[1], Hitoshi HORI[2] and Yasuo SHINOHARA[3]

1)Faculty of Pharmacy, Chiba Institute of Science, 3 Shiomi-cho, Choshi, Chiba, 288-0025; 2)Department of Biological Science and Technology, Faculty of Engineering, The University of Tokushima, 2-1 Minamijosanjima-cho, Tokushima 770-8506, 3)Institute for Genome Research, The University of Tokushima, 3-8 Kuramoto-cho, Tokushima 770-8503; Japan.

kohkura@sag.bekkoame.ne.jp

1. Introduction

ADP/ATP carrier (AAC) is the most abundant protein in mitochondrial carrier, catalyzes the exchange of ADP and ATP across the mitochondrial inner membrane (1-2). In the present study, we modeled the yeast type2 AAC (yAAC2) molecule based on bovine heart AAC1 (bAAC1) X-ray data and examined its molecular dynamics. The N- and C-terminus of yAAC2 are longer than those of bAAC1, and these additional region seems to concern with the AAC function (3). Previously, we reported the yAAC2 C-terminal role for nucleotide transport using C-terminal truncated yAAC2 mutants. Furthermore, the water accessibility of C-terminal region of yAAC2 had been examined using site-directed mutagenesis and chemical modification techniques. In this presentation, we modeled C-terminal truncated yAAC2 molecules and analyzed their molecular features for biological activity.

2. Method

Modeling of yeast AAC: Molecular modeling of yAAC2 was performed based on the PDB data of bAAC1 (1OKC) using insight II-discover. Minimization of modeled yAAC2 were performed using discover module under CVFF force field. Molecular dynamics analysis of minimized yAAC2 was performed, and obtained the yAAC2 conformers.

Structural Parameters of C-terminus: Molecular orbital (MO) analysis of C-terminal peptide (M304 ~ F317), which has crushed C-terminal Lys318 and it was deleted, of each MD conformer was performed using PM3. Electrostatic potential fields of C-terminal truncated yAAC2s were calculated, and the -1.0 kT/e contour was displayed as cloud. The various length of C-terminal peptides (M304 ~) were extracted from modeled (minimized) yAAC2, and their MO analysis were performed.

Function analysis of yAAC2: We prepared cDNAs encoding C-terminus-truncated mutants of yAAC2 by PCR. Biological responses of C-terminal truncated yAAC2 was examined using plate assay. For evaluation of viability of transformants on YPD or YPGly plates, culture suspensions were directly spread on the plates, which were subsequently incubated at 30 °C.

3. Results and Discussion

Modeling and Dynamics of yAAC2. Comparison of the amino acid sequence of yAAC2 with that of bAAC1 revealed that the N- and C-terminus of yAAC2 are 15- and 6-amino acids longer, respectively, than those of bAAC1 (Fig. 1).

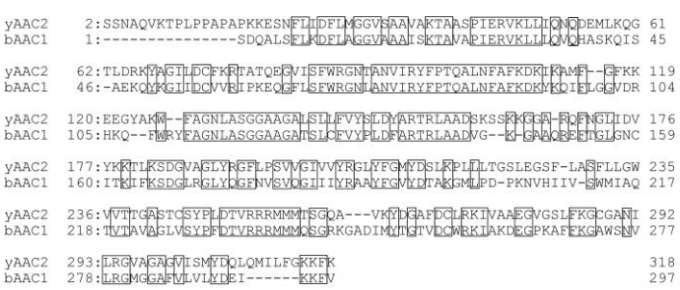
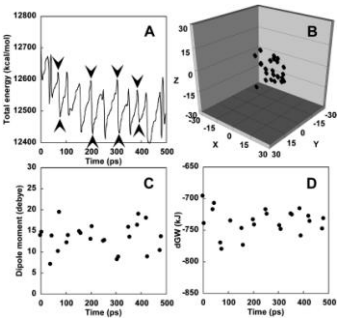


Fig. 1 Homology alignment of the amino acid sequences AAC2s. Fig. 2. Dynamic structure of modeled yAAC2 molecule.

The N- and C-terminal of yAAC2 molecule moved flexibly (light and dark gray structures in Fig. 2), while core region almost kept still. Total energy profile of yAAC2 during MD simulation fluctuated (12400 ~ 12770 kcal/mol) and gradually decreased (Fig. 3A). The C-terminal (M304 ~

F317) peptide dipole moments at maximal or minimal energy points, such as arrows in Fig. 3A, showed the similar direction (3B), and their intensities were 7.077 ~ 19.405 debye (3C). Solvation free energy of C-terminal peptides was changing at the range of -780.4 ~ -696.4 kJ (3D).



sample	YPD	YPGly
wt	+	+
WB-12	+	–
d2	+	+
d4	+	+
d5	+	+
d6	+	+
d7	+	–
d8	+	–
d9	+	–

Fig. 3. Molecular dynamics analysis of modeled yAAC2. Table I. Functional features of C-terminal truncated yAAC2 mutants.

yAAC2 C-terminal Biological Function and Electrostatic Potential Field. The 2 ~ 6 C-terminal amino acid truncated (d2 ~ d6) yAAC2 mutants transformed WB-12 cells were viable both on YPD and YPGly plates (Table I). The d7 ~ d9 mutants transformed cells were viable on YPD, but inviable on YPGly plate. Modeled yAAC2 expressed negative electrostatic potential field at C-terminal region (d0). The negative field always located at C-terminal area during C-amino acid truncation (d1 ~ d9), and the appearances were shown in d3, d6, d7 (Fig. 4). Further C-terminal truncation occurred the field invagination into core region, and the shapes were shown in d11, d14 and d16.

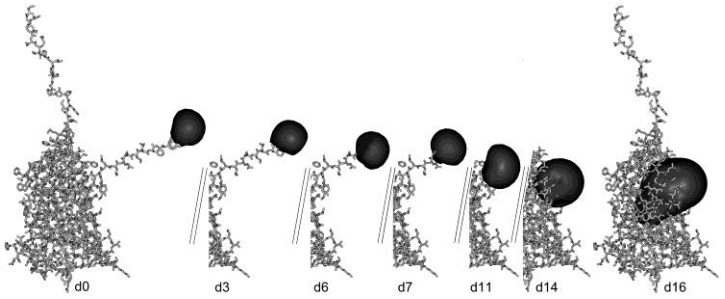


Fig. 4. Electrostatic potential field (ESP) of yAAC2.

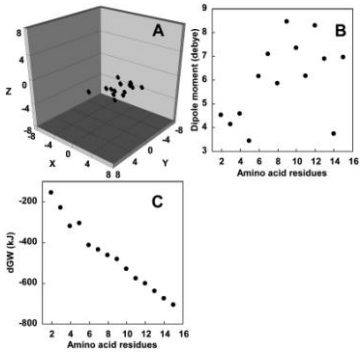


Fig. 5. Molecular orbital parameters of C-terminal peptides.

Molecular Features of yAAC2 C-terminal. Molecular features of C-terminal peptides (MY305, MYD306, MYDQ307, MYDQL308, MYDQLQ309, MYDQLQM310, MYDQLQMI311, MYDQLQMIL312, MYDQLQMILF313, MYDQLQMILFG314, MYDQLQMILFGK315, MYDQLQMILFGKK316, MYDQLQMILFGKKF317, MYDQLQMILFGKKFK318), which derived from the M304 ~ K318 region (Fig. 1), were examined using MO analysis. Dipole moments of these peptides directed almost same way (Fig. 5A), and their intensities tended to increase (3.427 ~ 8.446 debye) with amino acid length (5B). Stereo-hydrophobic parameter dGW indicated the increase of C-terminal peptide hydrophobicity with increase in amino acid length, and the dGW range was -708.5 ~ -156.7 kJ (5C).

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