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Functionally important conserved length of C-terminal regions of yeast and bovine ADP/ATP carriers, identified by deletion mutants studies, and water accessibility of the amino acids at the C-terminal region of the yeast carrier

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Abstract

Comparison of the amino acid sequence of yeast type 2 ADP/ATP carrier (yAAC2) with that of bovine type 1 AAC (bAAC1) revealed that the N- and C-terminus of yAAC2 are 15- and 6-amino acids longer, respectively, than those of bAAC1. In the present study, we focused on the difference in the C-terminal region between yAAC2 and bAAC1. Deletion of first six residues of C-terminus of yAAC did not markedly affect the function of yAAC2; however, further deletion of 1 amino acid (7th amino acid from the C-terminus) destroyed its function. On the contrary, deletion of the first amino acid residue of the C-terminus of bAAC1 caused failure of its functional expression in yeast mitochondria. Based on these results, we concluded that the 6-amino acid residue extension of the C-terminus of yAAC2 was not necessary for the function of this carrier and that the remainder of the C-terminal region of yAAC2, having a length conserved with that of bAAC1, is important for the transport function of AACs. We next prepared various single-Cys mutants in which each of 32 residues in the C-terminus of yAAC2 was replaced by a Cys residue. Since all mutants were successfully expressed in yeast mitochondria, we examined the reactivity of these cysteine residues with the membrane-impermeable sulfhydryl reagent eosin 5-maleimide (EMA). As a result, all cysteine residues that replaced the 9 continuous amino acids in Met^{310}-Lys^{318} showed high reactivity with EMA regardless of the presence of carboxyatractyloside or bongkrekic acid; and so this region was concluded to be exposed to the water-accessible environment. Furthermore, based on the reactivities of cysteine residues that replaced amino acids in the sixth transmembrane segment, the probable structural features of the C-terminal region of this carrier in the presence of bongkrekic acid were discussed.

Keywords: Mitochondria; ADP/ATP carrier; Mutants; Conformation change

Abbreviations: AAC, ADP/ATP carrier; bAAC1, bovine type 1 AAC; BKA, bongkrekic acid; CATR, carboxyatractyloside; EMA, eosin-5-maleimide; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; yAAC2, yeast type 2 AAC; y2NbAAC1, chimeric bAAC1 with the N-terminal amino acid sequence of yAAC2.

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1. Introduction

ADP/ATP carrier (AAC), the most abundant protein in the mitochondrial carrier family, catalyzes the exchange of ADP and ATP across the mitochondrial inner membrane (for reviews, see Klingenberg, 1989; Brandolin et al., 1993; Terada and Majima, 1997; Fiore et al., 1998; Nury et al., 2006). The AAC has been well established to show two conformational states, i.e., c- and m-state, in which the substrate recognition site faces cytosolic and matrix side, respectively, and to be fixed into the c-state or m-state by the addition of its specific inhibitor carboxyatractyloside (CATR) or bongkrekic acid (BKA), respectively. Furthermore, AAC is thought to show its function of transporting adenine nucleotides by interconversion between these two states.

The AAC consists of about 300-amino acid residues comprising three repeated domains. In an earlier study, the first model of the topology of AACs was proposed on the basis of hydropathy plots (Saraste and Walker, 1982). Since then, a number of experiments aimed at determining the topology of AACs have been performed with bovine mitochondria and submitochondrial particles by using photolabels (Boulay et al., 1983; Dalbon et al., 1988), chemical modifiers (Boulay and Vignais, 1984; Bogner et al., 1986), antipeptide antibodies (Brandolin et al., 1989), and proteolytic enzymes (Brandolin et al., 1989; Marty et al., 1992). As a result, the AAC was concluded to have a 6-transmembrane structure in the inner mitochondrial membrane, exposing its N- and C-terminus to the cytosolic side (Klingenberg, 1989; Majima et al., 1993; Hatanaka et al., 2001a). Recently, the crystal structure of bovine type 1 AAC (bAAC1) in complex with CATR was elucidated, and its predicted topology in the mitochondrial membrane was validated (Pebay-Peyroula et al., 2003). However, little is known about the dynamic molecular features of AAC, because its detailed structure in the m-state has not yet been clarified. Thus, studies on the structure/function relationship are required for elucidation of the molecular mechanism of nucleotide transport by AACs.

To examine this structure/function relationship of AAC, the use of the yeast *Saccharomyces cerevisiae* expression system has great advantage (Nelson et al., 1993; Hashimoto et al., 1999; De Marcos Lousa et al., 2002). In an earlier study, we attempted to express bAAC1 in the WB-12 strain of *S. cerevisiae*, which strain lacks the genes encoding type 1 and 2 AACs. However, trials to express bAAC1 itself in yeast cells were not successful. When we compared the amino acid sequence between yAAC2 and bAAC1, a marked difference was observed in their N-terminus; i.e., the N-terminus of yAAC2 is apparently longer than that of bAAC1. A chimeric bAAC1 mutant produced by replacing the N-terminus of bAAC1 with the corresponding region of yAAC2 (y2NbAAC1) was successfully expressed (Hashimoto et al., 1999). The reason why this replacement of N-terminal region of bAAC1 was effective for its expression in yeast cells was also further studied (Hatanaka et al., 2001b).

When we compared the amino acid sequence between yAAC2 and bAAC1, in addition to the differences in length and amino acid sequence in the N-terminus, those in its C-terminus were also remarkable. Thus, to examine the structural and functional properties of the C-terminal region of yAAC2, in the present study, we first prepared various C-terminal-truncated mutants of yAAC2 and y2NbAAC1 and examined their functional features. Furthermore, water accessibility of the C-terminal region of yAAC2 altered by site-directed mutagenesis and chemical modification was examined.

2. Materials and methods

2.1. Materials

The haploid strain of *S. cerevisiae* W303-1B (MATα *ade2-1, leu2-3, 112 his3-22, 15 trp1-1 ura3-1 can1-100*) was provided by Dr. Shimizu (Osaka University, Japan). The AAC-disrupted yeast strain, WB-12 (MATα *ade2-1 leu2-3, 112 his3-22, 15 trp1-1 ura3-1 can1-100 aac1; LEU2 aac2; HIS3*), and the single-copy type yeast shuttle vector pRS314-YA2P were prepared as described (Hashimoto et al., 1999). EMA and CATR were obtained from Molecular Probes (Eugene) and Sigma (St. Louis), respectively.

2.2. Preparation of DNA fragments encoding yAAC2 mutants

The cDNA fragment encoding yAAC2 was prepared by PCR using the primers HT499 and HT501, as described previously (Hatanaka et al., 2001a; Hashimoto et al., 1999). That encoding chimeric y2NbAAC1, in which the N-terminal region of bAAC1 was replaced by the corresponding region of yAAC2, was also prepared as described earlier (Hashimoto et al., 1999). Using these cDNAs as templates, we prepared cDNAs encoding C-terminus-truncated mutants of yAAC2 and y2NbAAC1 by PCR.

cDNAs encoding single-Cys mutants of yAAC2, having a single cysteine residue in its C-terminal region made by replacement of individual amino acids at Gly^{287}-Lys^{318} with cysteine, were prepared by overlap-extension PCR using a cDNA encoding Cys-less yAAC2 as a template (Hatanaka et al., 2001a).

Prepared cDNA fragments were subcloned into NdeI and BamHI sites of pRS314-YA2P having the promoter region of the yAAC2 gene, as described previously (Hashimoto et al., 1999); and the nucleotide sequences of DNAs encoding these mutants were confirmed. For expression of these mutants, prepared expression vectors were introduced into the AAC-disrupted yeast strain WB-12.

2.3. Culture condition of yeast cells

Yeast cells were grown at 30 °C in YP medium (1% yeast extract and 2% bactopeptone) supplemented with 2% glucose (YPD) or 3% glycerol (YPGly) or 2% galactose as a