CP12: a small nuclear-encoded chloroplast protein provides novel insights into higher-plant GAPDH evolution

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Abstract

Higher-plant chloroplast NAD(P)-glyceraldehyde 3-phosphate dehydrogenase (NAD(P)-GAPDH; EC 1.2.1.13) is composed of two different nuclear-encoded subunits, GAPA and GAPB, forming the highly active heterotetrameric A2B2 enzyme. The main difference between these two subunits is a C-terminal extension of about 30 amino acid residues of GAPB. We present cDNA clones for a nuclear-encoded chloroplast protein from pea, spinach and tobacco, which we have named CP12. The mature protein consists of only 74, 75 and 76 amino acid residues, respectively and contains two domains with significant homology to the C-terminal extension of GAPB. Affinity chromatography approaches reveal also a specific interaction between CP12 and chloroplast GAPDH. Northern blot analysis indicates that CP12 is, like plastid GAPDH, expressed in green and also in etiolated leaves. Further homology is observed between CP12 and ORF3, an open reading frame located in the hox gene cluster of Anabaena variabilis. This gene cluster encodes the subunits of the bidirectional NADP+-dependent [NiFeS] dehydrogenase. We propose therefore a common evolutionary origin of CP12 and higher-plant chloroplast GAPDH subunit GAPB from the cyanobacterial ORF3.

Introduction

GAPDH isozymes of Cyanobacteria and chloroplasts of green algae as well as all known cytosolic forms involved in glycolysis are composed of homotetramers of four identical subunits. In contrast, higher-plant chloroplasts contain two different nuclear encoded GAPDH subunits, GAPA (36 kDa) and GAPB (39 kDa). This enzyme forms a heterotetrameric (A2B2)4 complex (160 kDa) and can further reversibly aggregate to a higher complex (A2B2)6 decamer (600 kDa) [1, 2]. Some reports also suggest the presence of an A4 homotetrameric form (150 kDa) in chloroplasts of higher plants [2, 3, 4], while B4 homotetramers have never been observed.

The main difference between the higher-plant chloroplast GAPDH subunits GAPA and GAPB is a C-terminal extension of about 30 amino acid residues of GAPB [4, 5]. Limited proteolysis of the isolated (A2B2)4 complex from spinach with Staphylococcus aureus V8 protease totally removes the C-terminal extension of GAPB whereas subunit GAPA remains more or less intact. Without altering the enzymatic activity significantly, removal of the C-terminal extension leads to the disaggregation of the (A2B2)4 complex to yield the heterotetrameric (A2B2) form. This shows that this domain is exposed on the protein surface and that it is required for complex formation [6]. Although some effectors for the aggregation of the hexadecamer, such as NAD(P), and of its dissociation, such as NADPH, 1,3bisPGA, are known [7, 8], the detailed mechanisms of these reactions as well as the mechanisms for the assembly of the tetrameric GAPDH complexes in chloroplasts are still unknown. Physiological aspects concerning the reversible aggregation of the...
(A₂B₂) heterotetramer to the less active 'regulatory' (A₂B₂)₄ complex and the redox regulation of chloroplast GAPDH activity are summarized and discussed elsewhere (9, 10).

Sequence analysis of cDNAs for the three GAPDH species of tobacco has shown that the higher-plant chloroplast GAPDH subunits, GAPA and GAPB, are more related to each other than to the cytosolic subunit, GAPC. The chloroplast subunits, GAPA and GAPB, might therefore have evolved by a gene duplication of a common procaryotic ancestor different from that of the cytosolic subunit GAPC [3]. Further analysis of GAPDH sequences of a variety of species has confirmed that the two GAPDH subunits, GAPA and GAPB, of higher plants are closely related to the cyanobacterial Gap2 gene of Anabaena variabilis, whereas the cytosolic subunit, GAPC, pairs with the A. variabilis Gap1 gene [11, 12]. The origin of the C-terminal extension of higher-plant chloroplast GAPDH subunit, GAPB, still remains unclear.

We report here cloning and characterization of cDNAs for CP12 from pea, spinach and tobacco expression libraries, a nuclear-encoded chloroplast protein, that exhibits significant homology to the C-terminal extension of higher-plant chloroplast GAPDH subunit, GAPB. Coordinated gene expression of CP12 and chloroplast GAPDH as well as specific in vitro protein/protein interactions suggest a physiological role of CP12 in chloroplast GAPDH assembly and/or redox regulation. Furthermore, CP12 shows a significant overall homology to the open reading frame, ORF3, located in the hox gene cluster of A. variabilis. This gene cluster encodes the different subunits for the bidirectional NADP⁺-dependent [NiFeS] dehydrogenase. Sequence analysis together with the experimental data suggest that the evolution of CP12, most probably from the cyanobacterial ORF3, has enabled the development of higher-plant chloroplast GAPDH subunit GAPB.

Materials and methods

Plant growth

Spinach (Spinacia oleracea cv. Matador, used for in organello import and affinity chromatography was grown in a border. For northern blot analysis spinach was sowed in Vermiculite and kept in a growth chamber at 25 °C either in the dark or in a 12 h light/dark rhythm. Pea seedlings (Pisum sativum cv. Golf) were grown in the greenhouse under a 14/10 h light/dark regime.

Figure 1. Nucleotide and deduced amino acid sequences of the spinach cDNA for CP12. The nucleotide sequence is numbered relative to the adenine residue of the translation start codon. The putative processing site for the stromal peptidase is in bold letters.