PARTIAL SEPARATION AND INTERCONVERSION OF NADH- AND NADPH-LINKED ACTIVITIES OF PURIFIED GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE FROM SPINACH CHLOROPLASTS

Karlheinz PAWLIZKI and Erwin LATZKO

Institut für Chemie und Landwirtschaftliche Technologie Technische Universität München, 805–Weihenstephan, B.R.D.

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1. Introduction

Chloroplasts contain GAPDH* active with NAD(H) or NADP(H) in both directions of glycolysis [1,2], but efforts to completely separate the NAD(H) activity from the NADP(H) activity have so far not been successful [3–5].

We have now partially separated GAPDH into a high molecular weight form (MW 240 000) and a low molecular weight form (79 000) using chromatography on a Bio-Gel column. The higher molecular weight enzyme was active mainly with NADH while the lower molecular weight form was active predominantly with NADPH. Gel filtration in the presence of DTT resulted in conversion of high molecular weight GAPDH to a low molecular weight form. Both forms of GAPDH were composed of 2 different subunits (MW 42 000 and 39 000). In addition, during all gel filtrations a small protein factor able to activate chloroplast GAPDH was observed. These results are considered in relation to the regulation of chloroplast GAPDH during light-dark transitions.

* *Abbreviations*: GAPDH = glyceraldehyde 3-phosphate dehydrogenase EC 1.2.1.13; DTT = dithiothreitol; SDS = sodium dodecyl sulfate; NADH-(NADPH-)GAPDH = enzyme activity in the phostosynthetic direction; NAD – (NADP-) GAPDH = enzyme activity in the glycolytic direction.

2. Materials and methods

Chloroplast GAPDH was isolated from spinach leaves as described by Yonuschot et al. [4] with slight modifications. GAPDH activity was measured in an assay mixture containing 100 mM Tris-HCl pH 8.0; 10 mM $MgCl_2$; 10 mM GSH; 6 mM ATP; 0.2 mM NADH (or NADPH) and 2 units of 3-phosphoglycerate kinase. The reaction was started by the addition of 6 mM 3-phosphoglycerate. An enzyme unit was defined as 3.3 $\Delta A_{366\ nm}$ at 25°C.

The molecular weight of the enzyme was estimated according to the method of Andrews [6] using a Bio-Gel A 1.5 cm column (0.9 × 120 cm) with the following reference proteins: γ-globulin, albumin (bovinde) and myoglobin (horse). The void volume was determined with Dextran Blue 2000.

3. Results

Chloroplast GAPDH purified as described by Yonuschot et al. [4] was completely recovered in a single peak after chromatography through a DEAE-cellulose column. This preparation was then passed through a Bio-Gel A 1.5 cm column. Using this procedure the chloroplast GAPDH separated into two different peaks of activity (fig. 1A). The first peak contained most of the NADH-GAPDH but only a minor part of the applied NADPH-GAPDH while the second peak (fraction II) contained principally NADPH-GAPDH but only a trace of the applied NADH-GAPDH.

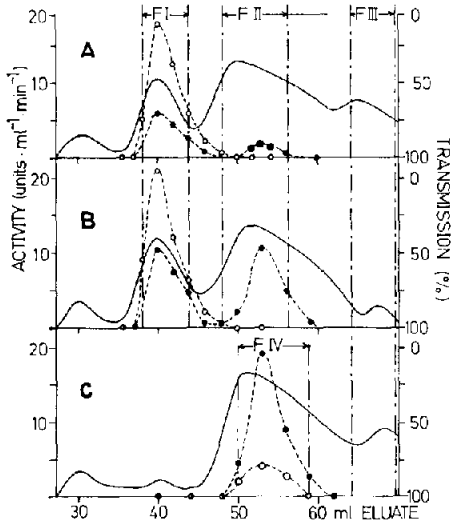

Fig. 1. Elution pattern of partially purified GAPDH on Bio-Gel A-1.5. Bio-Gel columns (0.9 × 120 cm) were equilibrated with buffer containing 0.1 M Tris–HCl pH 8.0; 10 mM EDTA and with the following additions: A: None; B: 0.03 mM NADP; C: 10 mM DTT. The partially purified GAPDH used was obtained by combining fractions containing activity from a DEAE-cellulose column.. ————, protein; o———o, NADH-linked activity; •— —• NADPH-linked activity.

During this procedure about 80% of the NADPH-GAPDH activity was lost but could be partially recovered by combining the two fractions I + II (table 1). Greater recovery of the initial activity was observed when a low molecular weight protein factor, eluted from the Bio-Gel column in a third peak (fraction III, fig. 1A), was added to the first two fractions (table 1), or when enzyme elution was performed in the presence of 0.03 mM NADP [10] (fig. 1B). No activation was observed when the first two fractions were assayed in the presence of egg albumin.

A quite different elution pattern from the Bio-Gel column was obtained when partially purified chloroplast GAPDH was eluted in the presence of 10 mM DTT (fig. 1C). In this case the protein peak of fraction I disappeared and the NADPH-GAPDH activity was eluted in only one peak (fraction IV) which corresponded to fraction II of fig. 1A. Fraction IV contained 20% of the applied NADH-GAPDH activity.

When the molecular weights of the different GAPDH fractions were determined fraction I had a molecular weight of 240 000 and both fractions II and IV had molecular weights of 79 000. These results could be explained if gel filtration, in the presence of DTT, resulted in a conversion of the high molecular weight GAPDH active with NADH to a low molecular weight enzyme active mainly with NADPH.

Fractions I and IV were checked for homogeneity and subunit composition by disc-gel-[7] and SDS-gel electrophoresis [8]. Disc-gel electrophoresis of

Table 1
Elution of purified chloroplast GAPDH from Bio-Gel A-1,5

Purification procedure	NADH linked		NADPH linked		Ratio NADH: NADPH
	Total units mg units protein^{-1}		Total units mg units protein^{-1}		
DEAE-cellulose column	146	14.6	241	24.1	1:1.7
Fraction I of Bio-Gel A-1,5 column (fig. 1)	126	30.0	50	12.0	1:0.4
Fraction II of Bio-Gel A-1,5	2	0.26	5.5	0.72	1:2.75
Fraction III of Bio-Gel A-1,5 column (figs. 1 and 3)	0	0	0	0	
I +II	132		119		1:0.9
I + II + III	132		178		1:1.35
Fraction IV of Bio-Gel A-1,5 column (fig. 3)	27	10.5	136	52.5	1:5.0
III + IV	34		181		1:5.3

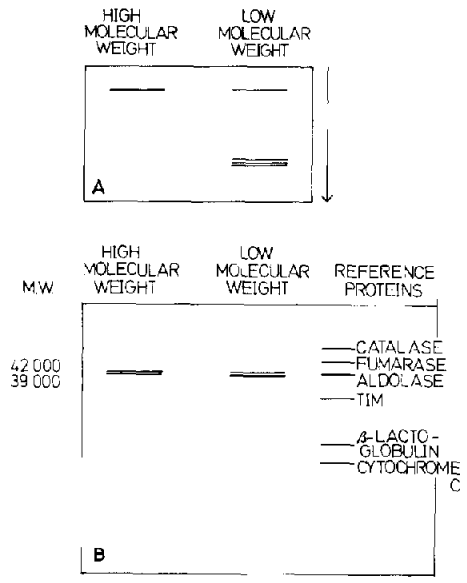

Fig. 2; A: Polyacrylamide disc gel electrophoresis of high and low molecular weight GAPDH. A: sample of 0.1 ml containing 40 μg of protein was used. B: SDS gel electrophoresis of high and low molecular weight GAPDH. A sample of 0.01 ml containing 20 μg of protein was applied. The known reference proteins were used to estimate the molecular weight of the two subunits of the different GAPDH forms.

fraction I showed only a single protein band and fraction IV revealed 4 bands (fig. 2A). The homogeneous protein of fraction I was divided into 2 different subunits by SDS-gel-electrophoresis with molecular weights of 42 000 and 39 000. The scanned protein bands showed a ratio of 1:1. The non-homogeneous protein of fraction IV revealed the same 2 subunits but in a ratio of $1: > 2$. (fig. 2B).

4. Discussion

Chloroplast GAPDH was separated into different forms with regard to molecular weight and activity with NADH vs. NADPH. The high molecular weight enzyme, predominantly active with NADH, could be converted into a pre-existent low molecular weight form more active with NADPH. All GAPDH forms contained the same subunits, but in different ratios. We suggest that the interconversion of chloroplast GAPDH observed may be related to an in vivo regulation during dark-light transitions. It is possible that, in darkness, GAPDH exists principally in an NADH-linked form comparable to fraction I, but in light a form most active with NADP (similar to fraction IV) predominates. On return to darkness all NADPH-linked enzyme may not be converted to NADH-linked enzyme and this could explain the appearance of fraction II (fig. 1A). In these experiments DTT would seem to simulate the dark-light transition but evidence for the light-dark transition has not yet been obtained.

Our observations agree with those of Müller and Ziegler [11] who found a 3–6-fold activation of NADPH-linked GAPDH in chloroplasts exposed to light and in DTT–treated homogenates stored in the dark. Anderson and Lim [9] have also reported changes in chloroplast GAPDH caused by DTT. They found that the enzyme from pea leaves was present in two electrophoretically distinct bands but after treatment with DTT only one band was observed.

Pupillo and Piccari [10] recently reported results for the conversion of chloroplast GAPDH between high and low molecular weight forms. They were able to convert high molecular weight enzyme (600 000, mainly active with NAD) to a low molecular weight form (145 000, mainly active with NADP) by gel filtration on Sephadex G-200 in the presence of 0.03 mM NADP but in contrast to our results DTT was not required for this conversion. In our experiments the elution profile of an NADP equilibrated column (fig. 1B) showed no significant difference to that without NADP (fig. 1A) except the NADPH-GAPDH of fraction II appeared about twice as active when NADP was added to the chromatography buffer. The function of the low molecular weight protein factor reported in our experiments is not yet clear; it may be that NADPH-GAPDH requires a protein factor to elicit full activity. Further investigations of this phenomenon are required.

Acknowledgements

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