The Presence of Malate Dehydrogenase in Thylakoids of *Anabaena cylindrica*, *Nostoc muscorum* and *Chlorogloeopsis fritschii*

A.-K. J. Sallal and N. A. Nimer

Department of Botany and Microbiology, Faculty of Science, Kuwait University, Kuwait

Z. Naturforsch. **45c**, 249–252 (1990); received July 4/October 13, 1989

Cyanobacteria, Malate Dehydrogenase, Thylakoids

The location of malate dehydrogenase in the cyanobacteria, *Anabaena cylindrica, Nostoc muscorum* and *Chlorogloeopsis fritschii* was investigated by the fractionation of cell-free extracts. The bulk of the enzyme activity was associated with the thylakoid membrane fraction, which also exhibited complete photosynthetic electron transport reactions. Malate dehydrogenase activity and photosystem II activities were inhibited by homologous antisera raised against isolated thylakoid membranes.

Introduction

In eukaryotic organisms isoenzymes of malate dehydrogenase are found in different subcellular compartments, the mitochondria [1, 2], chloroplasts [3, 4], glyoxysomes [5], peroxisomes [6, 7]. In cyanobacteria malate dehydrogenase functions as an enzyme in the tricarboxylic acid cycle [8, 9] and as an enzyme of the glycollate pathway [9, 10]. Kovatcheva and Bergman [11] have purified and characterized the enzyme from *Nostoc muscorum*. We have shown that malate dehydrogenase is largely associated with the thylakoid membranes of *Anacystis nidulans* [12]. Here, we report further on the localization of malate dehydrogenase using cell-free extracts of *Anabaena cylindrica, Chlorogloeopsis fritschii* and *Nostoc muscorum*.

Materials and Methods

Organisms and growth conditions

Anabaena cylindrica Lemm (strain CU 1403/2a), *Nostoc muscorum* (strain 1453/9) and *Chlorogloeopsis fritschii* (strain CU 1411/1) were obtained from the Culture Collection of Algae and Protozoa, Cambridge, England. They were grown in BG-11 medium [13]. Other growth conditions were as mentioned previously [12].

Abbreviations: DCPIP, 2,6-dichlorophenolindophenol; PS I (II), photosystem I (II).

Reprint requests to Dr. A.-K. J. Sallal.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/90/0300–0249 $ 01.30/0

Cell disruption and fractionation

The procedure for the production of thylakoid membranes was essentially that reported by Reuss [14] and Codd and Sallal [15]. Sucrose density gradient was done as described previously [12].

Malate dehydrogenase

The activity was assayed by following the initial rates of the decrease in the absorbance at 340 nm during the oxidation of the coenzyme (NADH) according to Codd and Stewart [10]. Average value for duplicate experiments were calculated with a standard deviation less than 3%.

Photosynthetic electron transport reactions

Ferricyanide-Hill reaction, photosystem I-Mehler reaction and photoreduction of NADP were carried out as reported by Sallal *et al.* [16]. Average value for duplicate experiments were calculated with a standard deviation less than 5%.

Chlorophyll and protein determination

Chlorophyll *a* was measured according to Kirk [17], protein was measured following the method of Lowry *et al.* [18].

Preparation of antisera

Antisera to washed thylakoid membranes of *A. cylindrica, N. muscorum,* and *C. fritschii* were prepared as described previously [12] using 1.2–2.0 mg proteins for each injection.

Results and Discussion

Malate dehydrogenase was detected in all fractions of all cyanobacteria investigated. The specific activities of the fractions obtained by differential centrifugation are shown in Table I. The highest specific activities were consistently exhibited by the 35,000 × *g* for 30 min pellet and washing of these sediment resulted in further increase in specific activity. Between 73–81% of the enzyme ac-

Table I. Distribution of malate dehydrogenase activity after differential centrifugation of extracts of cyanobacteria.

Fraction	*Anabaena cylindrica* Sp. act.[a]	% Act.	*Nostoc muscorum* Sp. act.	% Act	*Chlorogloeopsis fritschii* Sp. act.	% Act.
2,500 × *g* for 15 min supernatant	5.40	100	6.60	100	7.80	100
2,500 × *g* for 30 min pellet	0.30	8	0.50	6	0.21	5
supernatant	11.65	83	7.25	93	8.25	90
35,000 × *g* for 30 min pellet	14.60	80	14.40	87	12.70	85
supernatant	0.15	3	0.35	4	0.60	4
35,000 × *g* for 30 min washing of original 35,000 × *g* for 30 min pellet:						
pellet	15.20	73	15.60	81	13.40	75
supernatant	0.07	2	0.03	2	0.01	3

[a] Specific activity, μmol NADH · (mg protein)$^{-1}$ · h^{-1}.

Table II. Photosynthetic reactions of the washed 35,000 × *g* for 30 min pellet (thylakoids) fractions of the cyanobacteria.

Reaction	Specific activity *Anabaena cylindrica*	*Nostoc muscorum*	*Chlorogloeopsis fritschii*
Ferricyanide-Hill reaction[a]	248	250	220
PS I-Mehler reaction[b]	26	20	18
Photoreduction of NADP[c]	40	33	42

[a] Measured as the ferricyanide-Hill reaction with water as electron donor. Rates are given as μmol ferricyanide reduced · (mg chlorophyll)$^{-1}$ · h^{-1}.

[b] Measured as oxygen uptake in the presence of DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] using DCPIP/ascorbate donor couple and methylviologen as electron acceptor. Rates are given as μmol oxygen consumed · (mg chlorophyll)$^{-1}$ · h^{-1}.

[c] Measured as NADP reduced with water as electron donor and *via* photosystem II and I. Rates are given as μmol NADP reduced · (mg chlorophyll)$^{-1}$ · h^{-1}.

Table III. The effect of inhibitors on malate dehydrogenase in the 35,000 × *g* for 30 min pellet (thylakoids) fractions of the cyanobacteria.

Inhibitor[a]	Concentration	% Inhibition *A. cylindrica*[b]	*N. muscorum*[c]	*C. fritschii*[d]
Potassium cyanide	$1 \cdot 10^{-3}$ M	79	83	76
	$2 \cdot 10^{-4}$ M	18	21	15
Sodium azide	$1 \cdot 10^{-3}$ M	68	71	70
	$2 \cdot 10^{-4}$ M	15	19	17

[a] Inhibitors were added to the enzyme assay mixture to give the final concentrations used.

[b–d] Specific enzyme activities of *A. cylindrica*, *N. muscorum* and *C. fritschii* were 14.5, 16.7 and 14.0 μmol NADH · (mg protein)$^{-1}$ · h^{-1} respectively.

tivity, detected in the initial crude supernatant, remained associated with this membrane fraction after washing (Table I). All sucrose density gradient fractions of washed thylakoids of *A. cylindrica, N. muscorum* and *C. fritschii* were assayed for malate dehydrogenase activity and found that the highest enzyme activity correlated with the maximum chlorophyll *a* concentration. The same membrane fraction catalyzed the photoreduction of ferricyanide from water in the PS II-Hill reaction and the PS I-Mehler reaction using DCPIP/ascorbate couple as electron donor and methylviologen as electron acceptor. These pellets also catalyzed the photoreduction of NADP *via* PS II and PS I as presented in Table II. Enzyme activity was inhibited by potassium cyanide and sodium azide (Table III). A concentration of 10^{-3} M of potassium cyanide inhibited the enzyme activity in the thylakoid membranes of the tested cyanobacteria by 76–83%. However, sodium azide caused about 70% inhibition to the enzyme activity (Table III). The inhibition of the enzyme activity with these respiratory electron transport inhibitors raises the possibility that membrane-bound electron carriers may be involved. Homologous antisera to *A. cylindrica, N. muscorum* and *C. fritschii* caused 71–80% inhibition to the enzyme activity using intact thylakoid membranes (Table IV). However, after solubilization of the membranes with 1% (v/v) Triton X-100, complete inhibition of the enzyme was obtained (Table IV). In addition, pronase enzyme was added to *Anabaena cylindrica*

Table V. Malate dehydrogenase activity in intact thylakoid membranes of *A. cylindrica* treated with pronase enzyme.

	Specific activity[a]	% Activity
35,000 × *g* for 30 min original pellet (thylakoids)	14.2	100
35,000 × *g* for 30 min washings of thylakoids after treatment with pronase[b]		
First pellet	2.0	14.0
Second pellet	1.8	12.7

[a] Specific activity, μmol NADH · (mg protein)$^{-1}$ · h^{-1}.
[b] Pronase was added in a final concentration of 1 mg per ml and incubated with the membranes for 15 min at 8 °C.

Table IV. Effect of homologous antisera on PS II and malate dehydrogenase activity using washed 35,000 × *g* for 30 min pellet (thylakoids) fractions of the cyanobacteria.

Reaction	*Anabaena cylindrica* Sp. act.	% Inhibition	*Nostoc muscorum* Sp. act.	% Inhibition	*Chlorogloeopsis fritschii* Sp. act.	% Inhibition
Ferricyanide-Hill reaction of intact membranes[a]	253	0	250	0	243	0
+0.2 ml null serum	253	0	245	0	243	0
+0.2 ml homologous antiserum	0	100	0	100	0	100
Malate dehydrogenase activity of intact thylakoid membranes[b]	14.3	0	17.0	0	13.8	0
+0.2 ml null serum	14.3	0	17.0	0	13.8	0
+0.2 ml homologous antiserum	2.9	80	4.3	75	4.0	71
Malate dehydrogenase activity of solubilized thylakoid membranes[c]	14.5	0	17.3	0	14	0
+0.2 ml null serum	14.5	0	17.3	0	14	0
+0.2 ml homologous antiserum	0.0	100	0.0	100	0	100

[a] Photosystem II activity measured as ferricyanide photoreduction using water as electron donor. Specific activity expressed as μmol ferricyanide reduced · (mg chlorophyll)$^{-1}$ · h^{-1}.
[b] Specific activity measured as μmol NADH · (mg chlorophyll)$^{-1}$ · h^{-1}.
[c] Solubilization of the thylakoid membranes was by the addition of 1% (v/v) Triton X-100 to the final concentration.

thylakoid membranes to destroy all the accessible malate dehydrogenase and after washing of the membranes from pronase, the activity of malate dehydrogenase left inside the membranes was 12–14% as shown in Table V. This indicates that part of the enzyme is exposed to the surface thylakoid membranes while the other part is embedded inside these membranes. For comparison, the inhibitory effects of the homologous antisera on the ferricyanide-Hill reaction of the corresponding thylakoid membranes are also presented (Table IV).

Although the unicellular cyanobacterium *Gloeobacter violaceus* has been found to lack thylakoids [19], all other cyanobacteria examined, including those in this study, have been found to contain thylakoids for the performance of photosynthesis [20–22]. The association of respiratory electron transport with the thylakoid membranes was discussed in terms of the localization of glycollate dehydrogenase [15, 23, 24] and malate dehydrogenase [12] in cyanobacterial thylakoids. Cytochemical evidence on the function of cyanobacterial thylakoids, not only in photosynthesis but also as "mitochondrial equivalents" was reported by Bisalputra *et al.* [25]. Hatch and Slack [3] have reported evidence for the location of malate dehydrogenase in chloroplasts of maize leaves. This was also confirmed by the work of Ting and Rocha [4] who reported the association of malate dehydrogenase of green spinach leaves in the stroma of intact chloroplasts.

Acknowledgements

We would like to thank Prof. M. J. Merrett for reading the manuscript and for his valuable comments. This investigation was supported by Kuwait University, Research Council Grant SO 037.

[1] N.-S. Yang and J. G. Scandalios, Arch. Biochem. Biophys. **161,** 335–353 (1974).
[2] K. Miyatake, K. Washio, A. Yokota, Y. Nakano, and S. Kitaoka, Agric. Biol. Chem. **49,** 859–860 (1985).
[3] M. D. Hatch and C. R. Slack, Biochem. Biophys. Res. Commun. **34,** 589–593 (1969).
[4] I. P. Ting and V. Rocha, Arch. Biochem. Biophys. **147,** 156–164 (1971).
[5] R. W. Breidenbach, A. Khan, and H. Beevers, Plant Physiol. **43,** 705–713 (1968).
[6] R. K. Yamazaki and N. E. Tolbert, Biochim. Biophys. Acta **178,** 11–20 (1969).
[7] N. Collins and M. J. Merrett, Biochem. J. **148,** 321–328 (1975).
[8] J. Pearce, C. K. Leach, and N. G. Carr, J. Gen. Microbiol. **55,** 371–378 (1969).
[9] J. Pearce and N. G. Carr, J. Gen. Microbiol. **49,** 301–313 (1967).
[10] G. A. Codd and W. D. P. Stewart, Arch. Microbiol. **94,** 11–28 (1973).
[11] N. Kovatcheva and B. Bergman, Plant Sci. Lett. **16,** 189–194 (1979).
[12] A.-K. J. Sallal and N. A. Nimer, FEMS Microbiol. Lett. **50,** 151–155 (1988).
[13] R. Y. Stanier, R. Kunisawa, M. Mandel, and G. Cohen-Bazire, Bacteriol. Rev. **35,** 171–205 (1971).
[14] W. Reuss, Ph. D. Dissertation, Univ. of Köln, 1971.
[15] G. A. Codd and A.-K. J. Sallal, Planta **139,** 177–181 (1978).
[16] A.-K. J. Sallal, R. H. Al-Hasan, and N. A. Nimer, Planta **171,** 429–432 (1987).
[17] J. T. O. Kirk, Planta **78,** 200–207 (1967).
[18] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem. **193,** 265–275 (1951).
[19] R. Rippka, J. Waterbury, and G. Cohen-Bazire, Arch. Microbiol. **100,** 419–436 (1974).
[20] G. E. Fogg, W. D. P. Stewart, P. Fay, and A. E. Walby, The blue-green algae, Academic Press, New York, London 1973.
[21] D. W. Krogmann, in: The biology of blue-green algae (N. G. Carr and B. A. Whitton, eds.), pp. 81–99, Blackwell, Oxford 1973.
[22] N. G. Carr and B. A. Whitton, The biology of cyanobacteria, Blackwell Scientific Publications, London 1982.
[23] A.-K. J. Sallal and G. A. Codd, FEBS Lett. **56,** 230–234 (1975).
[24] B. Grodzinski and B. Colman, Plant Physiol. **58,** 199–202 (1976).
[25] T. Bisalputra, D. L. Brown, and T. E. Weier, J. Ultrastruct. Res. **27,** 182–197 (1969).