View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

CHARACTERIZATION OF THE DICYCLOHEXYLCARBODIIMIDE-REACTIVE PROTEIN FROM *RHODOSPIRILLUM RUBRUM* GROWN IN THE LIGHT AND IN THE DARK

R. SCHMID, H. H. KILTZ+, E. SCHNEIDER and K. ALTENDORF

Institut für Biochemie der Pflanzen, Abteilung Biologie, Ruhr-Universität Bochum and *+Institut für Biochemie, Abteilung Chemie, Ruhr-Universität Bochum, D-4630 Bochum, FRG*

Received 29 December 1980; revised version received 28 January 1981

1. Introduction

ATP synthetase (F_0F_1), the key enzyme in oxidative as well as in photophosphorylation, has been isolated and characterized from various sources [1]. In all cases, the enzyme complex is composed of two structurally-distinct entities. The F_1-part carries the catalytic centers of the enzyme, whereas the membrane-integrated F_0 moiety is thought to form a proton channel.

One of the polypeptides belonging to the F_0-part is known to bind covalently the specific inhibitor dicyclohexylcarbodiimide (DCCD); this DCCD-reactive protein has been isolated and well characterized from different organisms [2–6] as well as from a purified ATP synthetase preparation [7].

As a facultative photosynthetic bacterium *Rhodospirillum rubrum* is capable of both photoheterotrophic and aerobic growth. In the first case the bacterium develops chromatophores, containing an ATPase complex, isolated and characterized in [8–10]. Like F_0F_1 preparations from other sources, it translocates protons and is capable of synthesizing ATP in the presence of bacteriorhodopsin [11] when incorporated into liposomes. The question arises whether the same ATPase complex takes part in oxidative phosphorylation when the bacterium is grown under aerobic conditions. In [12–14] evidence has been obtained that the F_1 moieties, isolated from both dark-grown and photosynthetically-grown cells of *Rhodopseudomonas capsulata* are identical.

Here, we report that the DCCD-reactive protein from *R. rubrum* grown in the dark is identical to that grown in the light. This suggests that also the F_0 parts from both ATPase complexes are identical. We also report that the *R. rubrum* protein, in contrast to all other DCCD-binding proteins isolated so far [2] contains tryptophan.

2. Materials and methods

Rhodospirillum rubrum FR 1 cells were grown under photoheterotrophic conditions as in [15]. Aerobic cells were a generous gift from Dr J. Oelze (Freiburg). Chromatophores were prepared by ultrasonication as in [15]. Cytoplasmic membranes from aerobic cells were prepared as in [16]. The ATPase complex was purified from chromatophores as in [9]. For labeling with [^{14}C]DCCD, chromatophores or membrane vesicles were incubated in 0.2 M glycylglycine (pH 7.5), 50% glycerol, 2 mM dithiothreitol, 5 mM $MgSO_4$, at 9 mg protein/ml with 100 μM [^{14}C]-DCCD for 24 h at 0°C. After centrifugation the vesicles were washed twice with 10 mM glycylglycine (pH 7.5). In the case of whole cells or membranes the DCCD-reactive protein was isolated as in [17]. When the purified ATPase complex was used as starting material, the protein was isolated according to [7].

Amino acid analysis was performed as in [17]. Protein was determined as in [18]. For protein dissolved in organic solvents a modification of the described method was used [19]. In some cases amino acid analysis was applied for protein determination. SDS gel electrophoresis in the presence of 8 M urea was carried out as in [20]. Unlabeled dicyclohexylcarbodiimide was obtained from Sigma (St Louis, MO). [^{14}C]DCCD was synthesized as in [20].

3. Results

To follow the purification of the DCCD-reactive protein from *Rhodospirillum rubrum*, membrane ves-

Table 1
Purification of the DCCD-binding proteins from *Rhodospirillum rubrum* grown in the light and in the dark

	Light-grown		Dark-grown	
	Protein (mg)	[^{14}C]DCCD bound (nmol/mg protein)	Protein (mg)	[^{14}C]DCCD bound (nmol/mg protein)
Membrane vesicles	351 (100%)	1.3	650 (100%)	0.9
Crude proteolipid[a]	3.9 (1.1%)	5.9	2.6 (0.4%)	14.8
Purified protein	0.21 (0.06%)	10.9	0.52 (0.08%)	23.4

[a] The proteolipid was extracted with chloroform:methanol (2:1) and precipitated with ether

icles were labeled with [^{14}C]DCCD. For chromatophores as well as for membrane vesicles prepared from aerobically-grown cells, the radioactivity was enriched in the preoteolipid (table 1). At a first glance the factor of enrichment is rather low. However, we observed that in the membranes a substantial amount of radioactivity was incorporated unspecifically without being bound to protein, thereby enhancing the apparent initial activity. Further purification of the crude proteolipids was achieved by subsequent chromatography on CM-cellulose [17]. For the ^{14}C-labeled protein from aerobically grown cells the elution profile is shown in fig.1. The protein was eluted from the column by chloroform:

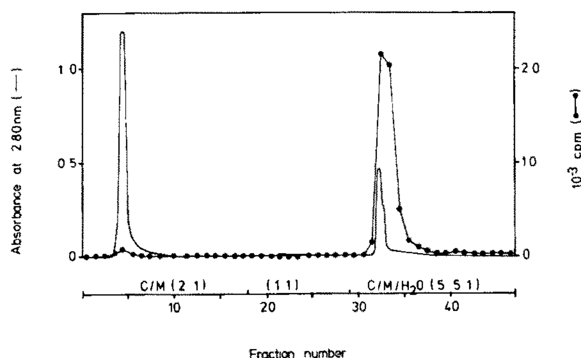

Fig.1. CM-cellulose chromatography of the crude proteolipid extracted from [^{14}C]DCCD labeled membrane vesicles from aerobically (dark) grown cells of *Rhodospirillum rubrum*. Crude proteolipid (10 mg) dissolved in chloroform:methanol (2:1) was loaded on a column (1 × 25 cm) pre-equilibrated with chloroform:methanol (2:1). The column was washed with 5 vol. chloroform:methanol (2:1) and then with 3 vol. chloroform:methanol (1:1). The protein was eluted with chloroform:methanol:H_2O (5:5:1) at a flow rate of 25 ml/h: (——) absorbance at 280 nm; (•——•) radioactivity of [^{14}C]-DCCD.

methanol:water (5:5:1) in a single peak containing ~20% of the applied protein. The radioactivity bound did not increase in a parallel way (table 1). The reason for this is probably that under our conditions other proteins were labeled by DCCD. For the DCCD-reactive protein from chromatophores this effect was even more pronounced: After elution of the DCCD-reactive protein a greenish peak was eluted from the column by 100 mM ammonium-acetate in chloroform:methanol:water (5:5:1), which contained protein associated with more than half of the radioactivity applied (not shown). Subsequently, the same protocol was used to prepare larger amounts of both proteins in the unlabeled form. Analysis by SDS gel electrophoresis in the presence of 8 M urea showed that the isolated proteins migrated as single bands with identical R_F-values (fig.2). In addition, the same result was obtained by extracting the protein from a purified F_0F_1 complex from chromatophores (fig.2C). Gels of the radioactivity labeled proteins from chromatophores as well as from aerobically-grown cells were sliced and the radioactivity was counted. Practically all radioactivity was found in a single peak coinciding with the stained band (not shown).

All 3 proteins were further characterized by amino acid analysis (table 2). The amino acid composition of the DCCD-binding proteins was almost identical. The slight differences found, especially for alanine, leucine, and isoleucine may be due to the limited hydrolysis time used in the case of the chromatophore proteins. Stable values for those amino acids were only obtained after 96 h hydrolysis as revealed by a time-dependence study. The integers which are closest to the extrapolated values sum up to a polypeptide of 75 residues with M_r 7500. In accordance with this value, the R_F-values for all 3 proteins from *Rhodospirillum rubrum* were in the same range as

Fig.2. SDS–urea gel electrophoresis of the DCCD-binding protein of different materials from *Rhodospirillum rubrum* after staining with Coomassie brilliant blue: (A) aerobically (dark) grown cells; (B) chromatophores; (C) F_0F_1 from chromatophores.

that found for the DCCD-binding protein from *E. coli* in the same gel system (M_r 8288, [17]).

Based on this M_r-value under our conditions 38% of the protein was labeled by [^{14}C]DCCD with light-grown cells and 33% of the protein with dark-grown cells.

The hydrophobic nature of the proteins is clearly reflected by the high content of apolar amino acids.

Table 2
Amino acid composition of the DCCD-reactive protein from different materials of *Rhodospirillum rubrum*

Amino acid	Aerobically grown cells[a]		Chromatophores[b] (molar ratios)	F_0F_1 from chromatophores[b]
Asp	4.2	(4)	4.2	4.2
Thr	2.9	(3)	3.1	2.5
Ser	2.0	(2)	2.3	2.7
Glu	3.1	(3)	3.6	3.6
Pro	1.0	(1)	1.4	1.7
Gly	10.1	(10)	10.2	10.2
Ala	17.1	(17)	15.7	14.9
Cys	–[c]		n.d.	n.d.
Val	6.0	(6)	5.4	5.4
Met	2.8[d]	(3)	2.1	2.6
Iso	8.9	(9)	7.4	6.3
Leu	8.3	(8)	6.8	6.1
Tyr	1.1	(1)	1.2	1.2
Phe	3.0	(3)	3.4	3.0
Try	1.6[e]	(2)	1.4[e]	n.d.
Lys	2.2	(2)	2.0	1.7
Arg	1.1	(1)	1.2	1.6
Total		75		

[a] Values extrapolated from 24, 48, 72, 96 and 180 h hydrolysis
[b] Values from 48 h hydrolysis
[c] Determined after performic acid oxidation [21]
[d] Determined as methioninesulfone [21]
[e] Determined after hydrolysis in mercaptoethanesulfonic acid [22]

n.d., not determined

Most abundant are alanine, glycine, isoleucine and leucine. Cysteine and histidine are absent. The presence of tryptophan is confirmed by the UV-spectrum of the protein (not shown).

4. Discussion

Incubation of chromatophores or membrane vesicles from aerobically-grown cells from *R. rubrum* with [^{14}C]DCCD results in the labeling of hydrophobic proteins. According to the data in [8] >90% inhibition of the ATPase activity of chromatophores can be expected applying the concentration of DCCD used.

Several proteins from chromatophores of *R. rubrum* are solubilized by organic solvents [23]. We have evidence that more than one of those proteins were labeled by DCCD under our conditions. However, based on the following arguments we conclude that the purified DCCD-binding protein is a component of the membrane-integrated F_0-part of the ATPase complex.

(i) We extracted with chloroform/methanol a protein from an intact F_0F_1 preparation of chromatophores which has the same amino acid composition and electrophoretic mobility as the protein isolated directly from the two kinds of membrane vesicles.

(ii) The amino acid composition of the protein from *R. rubrum* basically resembles the DCCD-binding proteins isolated from other organisms [2]. Striking similarities are the high content of glycine, alanine, leucine and isoleucine, the low content of polar basic amino acids and the absence of histidine. However, there are two important differences: the DCCD-binding protein from *R. rubrum* is the first one investigated so far in which the content of isoleucine is higher than that of leucine and, most strikingly, in which tryptophan is present.

Our results show that the DCCD-binding proteins isolated from *R. rubrum* grown anaerobically in the light or aerobically in the dark have the same amino acid composition. This in itself strongly suggests that also their primary structure is identical; however, definite proof of this will have to be supplied by amino acid sequencing.

Taken together, these results lend support to the notion [24] that the chromatophores arise from the cytoplasmic membrane and that this membrane is the locus for both oxidative and photophosphorylation. It has already been shown by reconstitution experiments that the F_1-part of the ATPase complex in *R. capsulata* is interchangeable between both kinds of membranes [12,13]. Additional immunological experiments have provided further evidence for the identity of this part of ATPase in both membranes [14]. On the other hand, our experiments demonstrate the structural identity of one component within the membrane-integrated F_0-part of the ATPase.

Acknowledgements

The authors wish to thank Mrs E. Hoffmann-Posorske for excellent technical assistance, Dr J. Oelze for the generous gift of aerobically grown cells and Dr T. Bakker-Grunwald for a critical revision of the manuscript. This work was supported in part by the Deutsche Forschungsgemeinschaft, by the Ministerium für Wissenschaft und Forschung (Nordrhein-Westfalen) and by the Fonds der chemischen Industrie.

References

[1] Baird, B. A. and Hammes, G. G. (1979) Biochim. Biophys. Acta 549, 31–53.
[2] Sebald, U. and Wachter, E. (1978) in: Energy Conservation in Biological Membranes (Schäfer, G. and Klingenberg, R. eds) pp. 228–236, Springer-Verlag, Berlin, New York.
[3] Nelson, N., Eytan, E., Notsani, R., Sigrist, M., Sigrist-Nelson, K. and Gitler, C. (1977) Proc. Natl. Acad. Sci. USA 74, 2375–2378.
[4] Wachter, E., Schmid, R., Deckers, G. and Altendorf, K. (1980) FEBS Lett. 113, 265–270.
[5] Hoppe, J., Schairer, H. U. and Sebald, W. (1980) FEBS Lett. 103, 107–111.
[6] Hoppe, J. and Sebald, W. (1980) Eur. J. Biochem. 107, 57–65.
[7] Sone, N., Yoshida, M., Hirata, H. and Kagawa, S. (1979) J. Biochem. (Tokyo) 85, 503–509.
[8] Schneider, E., Müller, M. W., Rittinghaus, K., Thiele, V., Schwulera, U. and Dose, K. (1979) Eur. J. Biochem. 97, 511–517.
[9] Schneider, E., Friedl, P., Schwulera, U. and Dose, K. (1980) Eur. J. Biochem. 108, 331–336.
[10] Bengis-Garber, P. and Gromet-Elhanan, Z. (1979) Biochemistry 18, 3577–3581.
[11] Müller, M. W., Schneider, E., Baltscheffsky, M. and Dose, K. (1978) Hoppe-Seyler's Z. Physiol. Chem. 359, 1123–1124.
[12] Melandri, B. A., Baccarini-Melandri, A., San Pietro, A. and Gest, H. (1971) Science 174, 514–516.
[13] Baccarini-Melandri, A. and Melandri, B. A. (1972) FEBS Lett. 21, 131–134.
[14] Lien, J. and Gest, H. (1973) Arch. Biochem. Biophys. 159, 730–737.
[15] Schneider, E., Schwulera, U., Müller, H. W. and Dose, K. (1978) FEBS Lett. 87, 257–260.
[16] Oelze, J. (1976) Biochim. Biophys. Acta 436, 95–100.
[17] Altendorf, K., Hammel, U., Deckers, G., Kiltz, H. H. and Schmid, R. (1979) in: Functional and Molecular Aspects of Biomembrane Transport (Klingenberg, E. M. et al. eds) pp. 54–61, Elsevier/North-Holland, Amsterdam, New York.
[18] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[19] Altendorf, K. and Zitzmann, W. (1975) FEBS Lett. 59, 268–272.
[20] Altendorf, K. (1977) FEBS Lett. 73, 271–275.
[21] Hirs, C. W. H. (1967) Methods Enzymol. 2, 59–62.
[22] Penke, B., Ferenczi, R. and Kovacs, K. (1974) Anal. Biochem. 60, 45–50.
[23] Tonn, S. J., Gogel, G. E. and Loach, P. A. (1977) Biochemistry 16, 877–885.
[24] Oelze, J. and Drews, G. (1972) Biochim. Biophys. Acta 265, 209–239.