

The molecular structure of the Na⁺-translocating F₁F₀-ATPase of *Acetobacterium woodii*, as revealed by electron microscopy, resembles that of H⁺-translocating ATPases

Jutta Reidlinger**, Frank Mayer, Volker Müller*

Institut für Mikrobiologie der Georg-August-Universität, Grisebachstr. 8, D-37077 Göttingen, Germany

Received 20 October 1994

Abstract The Na⁺-translocating F₁F₀-ATPase of *Acetobacterium woodii* was examined by electron microscopy. After reconstitution into proteoliposomes, knobs typical for the F₁ domain were visible on the outside of the membrane. The F₁-part of the isolated enzyme showed a hexagonal symmetry suggesting an $\alpha_3\beta_3$ structure, and the F₁F₀ complex had molecular dimensions very similar to those of H⁺-translocating ATPases of *E. coli*, chloroplasts, and mitochondria.

Key words: F₁F₀-ATPase (EC 3.6.1.34); Na⁺-translocating; Electron microscopy; Acetogenic bacteria

1. Introduction

Most acetogenic bacteria are able to derive energy from the conversion of 2 mol CO₂ and 4 mol H₂ to acetate via the acetyl-CoA (Wood) pathway. This reaction sequence, which is not coupled to net ATP formation by substrate level phosphorylation [1], is strictly dependent on sodium ions in some of these organisms, e.g. *Acetobacterium woodii*, *Acetogenium kivui*, and *Peptostreptococcus productus* [2–4]. In *A. woodii*, a primary sodium ion pump is present which couples one of the reactions leading from methylene tetrahydrofolate to acetyl-CoA with the extrusion of sodium ions [2,5]. The Na⁺ gradient is then used for ATP synthesis (Fig. 1) [6]. The ATPase has been purified and characterized as a Na⁺-translocating F₁F₀-ATPase [7]. The catalytic properties of this enzyme are very similar to those of the Na⁺-translocating F₁F₀-ATPase of *Propionigenium modestum* [8–10]. Although several biochemical and molecular data clearly indicated that the Na⁺-translocating ATPase from *A. woodii* belongs to the F₁F₀-ATPases ([7], A. Forster, R. Daniel, and V. Müller, manuscript in preparation) the enzyme differs from most bacterial F₁F₀-ATPases in three respects: (i) according to SDS-PAGE and N-terminal sequencing of the subunits, the enzyme contains only 6 instead of 8 subunits, (ii) the F₁ part of the enzyme could not be removed from membrane vesicles by standard procedures like treatment with low ionic strength solutions or chloroform, and (iii) it is inhibited by nitrate [7]. To determine whether this difference is reflected in a different architecture of the enzyme, we started an investigation on the ultrastructure of this enzyme by electron microscopy. This study demonstrates that the macromolecular organization of the ATPase from *A. woodii* exactly resembles that of other F₁F₀-ATPases, including the well-characterized enzymes from *E. coli* and mitochondria.

2. Materials and methods

2.1. Organism and growth conditions

Acetobacterium woodii (DSM 1030) was grown under anaerobic conditions with 20 mM fructose as the carbon source as described [7].

2.2. Purification of ATPase

ATPase of *A. woodii* was purified as described [7]. In short, inverted vesicles were prepared by use of a French pressure cell after protoplasting the cells with lysozyme. From washed vesicles the enzyme was purified by solubilization with Triton X-100, precipitation with polyethylene glycol (PEG)-6000 and subsequent gel-filtration. ATPase activity was monitored by the continuous assay of Penefsky and Bruist [11] with the modifications described [7]. All protein determinations were done by a modified Lowry procedure [12]. Triton X-100 was removed by the method of Tornqvist and Belfrage prior to the protein determination [13]. A typical purification starting from 25 g cells (wet mass) yielded 15 mg pure enzyme with a specific activity of 15–20 U/mg protein.

2.3. Reconstitution into proteoliposomes

Prior to reconstitution experiments, crude phosphatidylcholine type II-S was purified according to Kagawa and Racker [14]. The incorporation of the purified enzyme into preformed proteoliposomes was carried out by detergent dialysis as described [7], except that 20 mM Tris-HCl, pH 7.5, 5 mM MgCl₂, was used throughout the reconstitution procedure. More than 95% of the activity was found in the pellet when these proteoliposomes were centrifuged after the dialysis (200,000 × g, 60 min, 4°C), indicating almost complete reconstitution into the proteoliposomes.

2.4. Electron microscopy

For detergent removal, the ATPase was diluted with 150 mM Tris-HCl, pH 7.5, 1.5 mM MgCl₂, to a final protein concentration of 50 µg/ml and dialyzed for 48 h. Proteoliposomes were diluted with 20 mM Tris-HCl, pH 7.5, 5 mM MgCl₂, to a protein concentration of 0.1 mg/ml. Both samples were negatively stained with an aqueous uranyl acetate solution (4% w/v, pH 4.8) by the diffusion technique of Valentine et al. [15]. Micrographs were prepared with a Philips EM 301 electron microscope operated at 80 keV in the conventional transmission mode, at calibrated magnifications ranging from 38,000 × to 70,000 ×. Measurements of particle size were made from prints at a magnification of 420,000 ×.

3. Results

Proteoliposomes containing the purified ATPase of *A. woodii* were shown to catalyze an ATP-dependent transport of Na⁺ [7].

*Corresponding author. Fax: (49) (551) 393 793.
E-mail: WMUELLE2@gwdgvl1.dnet.gwdg.de

**Present address: Department of Biochemistry, Adrian Building, University Road, Leicester, LE1 7RH, UK.

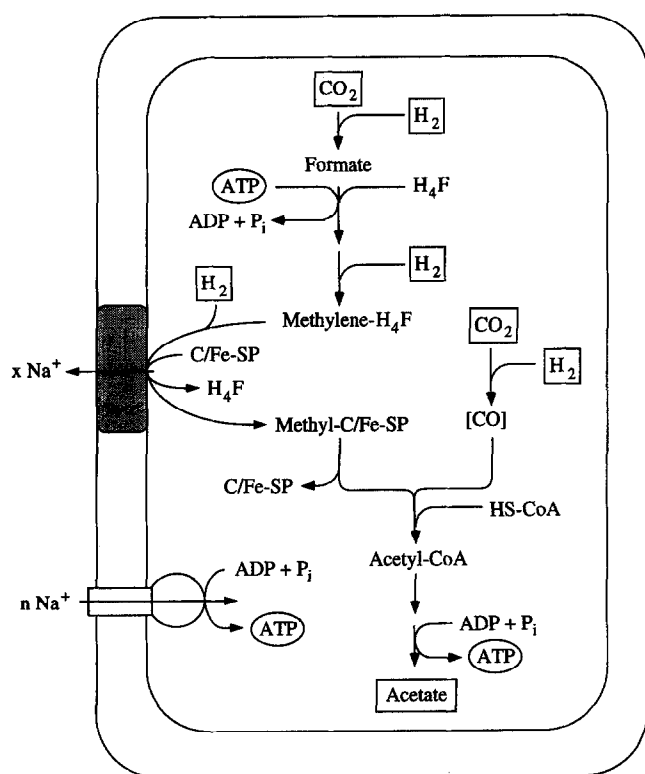


Fig. 1. Bioenergetics of acetogenesis from $H_2 + CO_2$ as carried out by *Acetobacterium woodii*. H_4F , tetrahydrofolate; C/Fe-SP, corrinoid/iron-sulfur protein. Please note that Na^+ extrusion as shown is coupled to the reaction sequence leading from methylene- H_4F to a methylated intermediate; the actual sodium ion pump is not known yet.

←

An electron micrograph of these proteoliposomes is shown in Fig. 2. The size of the vesicles in this preparation is rather inhomogeneous, with diameters ranging from 15 to 150 nm. Clearly visible at the periphery of many vesicles are knob-like structures typical of F_1 particles. The number of these ATPase particles per vesicle varied from 0 to 6. The F_1 particles of the proteoliposomes had diameters between 9 and 12 nm, values which are similar to the diameter obtained for F_1 -ATPases from other sources [16–18].

Further studies on the ultrastructure of the ATPase from *A. woodii* were done with the purified F_1F_0 complex in the absence of phospholipids and detergents. Micrographs of this preparation are shown in Fig. 3. Due to the absence of any hydrophobic components in the buffer, the F_0 parts of the enzyme aggregated into chains or spheres of 2–15 ATPase complexes. The overview in Fig. 3 shows that the ATPase sample used for the electron microscopic studies was homogeneous and that more than 90% of the particles represented intact F_1F_0 -ATPases, which is in accordance with the high recovery

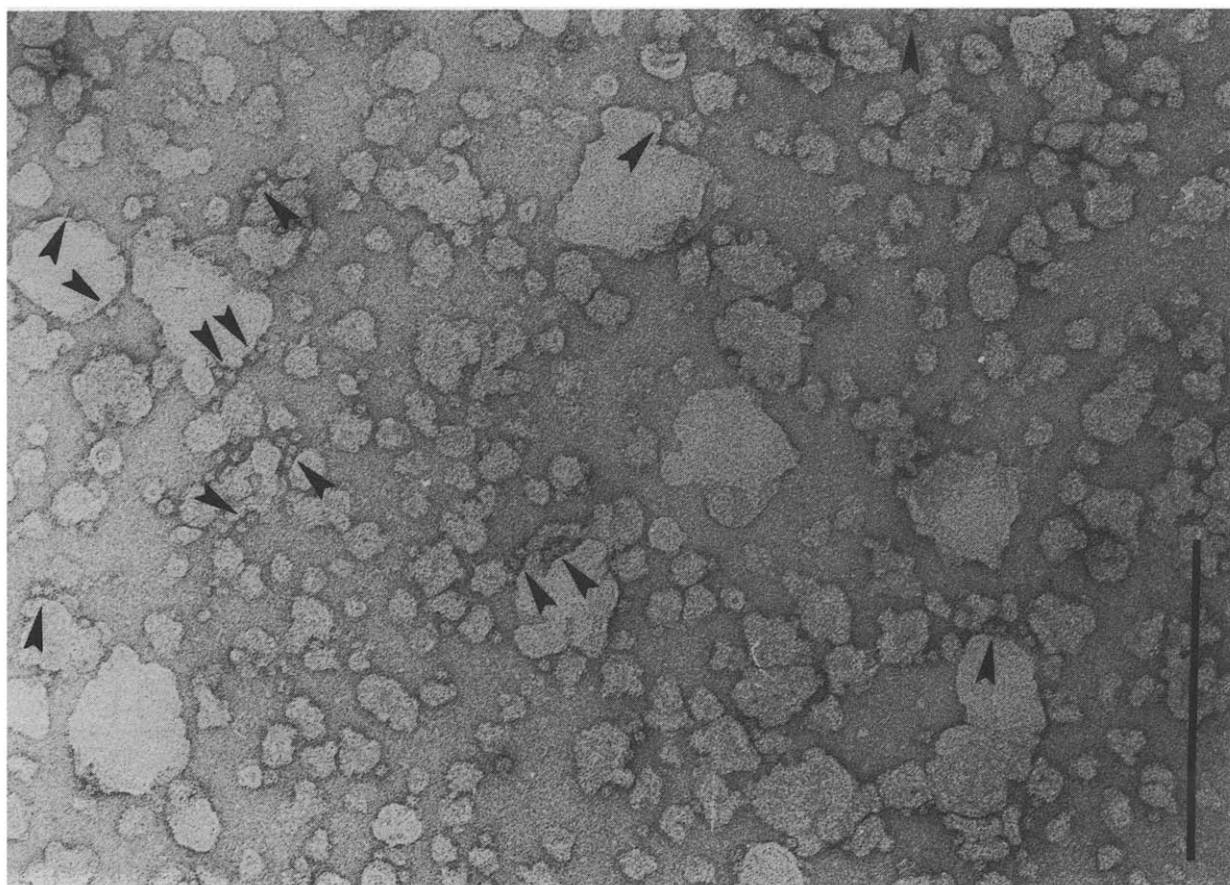


Fig. 2. Proteoliposomes containing the purified ATPase of *A. woodii* after negative staining with uranyl acetate. The arrowheads indicate the F_1 parts of ATPase enzymes incorporated into the vesicles. Bar = 300 nm.

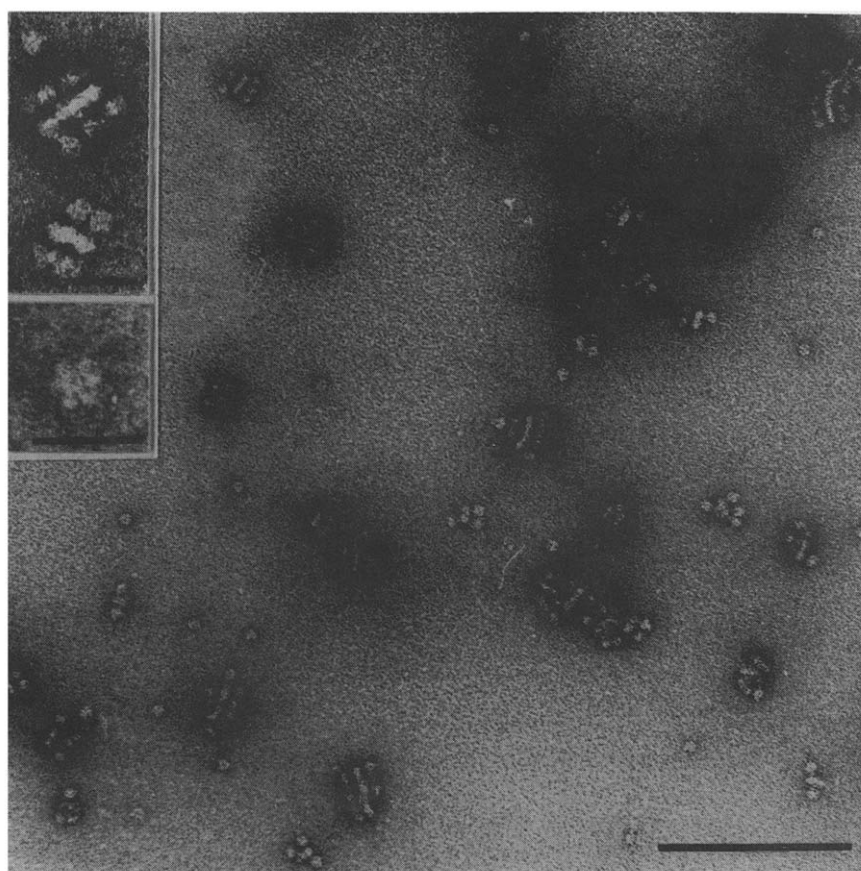


Fig. 3. F_1F_0 -ATPase from *A. woodii* after negative staining with uranyl acetate. The upper inset shows a side view of an enzyme aggregate, the lower inset a face view of an isolated F_1 part of the enzyme. Bar = 200 nm for the overview and 25 nm for both insets.

of ATPase activity upon centrifugation of proteoliposomes and the high degree of inhibition by DCCD observed in earlier studies [7]. The upper inset in Fig. 3 shows a side view of some F_1F_0 particles. The F_1 and the F_0 parts of the enzymes exhibited distinct structures; they appeared to be connected by a small stalk. While the F_1 parts of these particles could be visualized as distinct domains with a diameter of 10–11 nm, the F_0 domains of individual ATPase molecules within an aggregate could not be distinguished due to the hydrophobic interactions

of adjacent moieties. The lower inset in Fig. 3 depicts a projection of an isolated F_1 particle shown very close to face view. This projection reveals 6 intensity maxima arranged in a circle around a central mass. Since the structure displays a sixfold symmetry, an average interval of 60° can be anticipated for the peripheral masses. The molecular dimensions of these particles are summarized in Table 1; they are very similar to those of F_1F_0 -ATPases from other bacteria, mitochondria and chloroplasts; a finding which corroborates the structural relationship of the *A. woodii* ATPase with other F_1F_0 -ATPases.

Table 1

Comparison of the molecular organization of ATPase from *A. woodii* with ATPases from mitochondria, chloroplasts, and *E. coli* (data taken from [17] and [18])

model of	ATPase from	ATPases from		
F_1F_0 -ATPases	<i>A. woodii</i>	mitochondria	chloroplasts	<i>E. coli</i>
	[nm]	[nm]		
	a: 20.5 ± 0.6	a: 22.5 ± 2.2	20.3 $\pm$ 2.5	19.0
	b: 9.1 ± 0.8	b: 9.0 ± 0.8	8.3 $\pm$ 1.0	8.5
	c: 4.2 ± 0.8	c: 4.6 ± 0.6	3.7 $\pm$ 0.7	3.7
	d: 7.8 ± 0.8	d: 8.9 ± 0.8	8.3 $\pm$ 0.8	6.8
	e: 10.5 ± 0.6	e: 11.7 ± 0.9	11.0 $\pm$ 1.1	10.1

4. Discussion

Despite the biochemical differences, most notably the different subunit composition, the Na^+ -translocating F_1F_0 -ATPase of *A. woodii* appears to have the same molecular architecture as other bacterial ATPases, namely containing 8 subunits. When compared with previous studies on ATPases from different sources depicted by electron microscopic techniques similar to those applied in the present study [19–21], the micrographs of the ATPase from *A. woodii* strongly suggest that the peripheral masses of the F_1 part of the complex also consist of alternating α and β subunits, while the central mass is formed, at least, by the γ and ϵ subunit. Whether this central mass also contains an homologue of subunit δ cannot be answered at the moment. After all, it is not yet clear whether the enzyme contains a subunit δ homologue at all [7]. However, the presence

or absence of a subunit δ homologue does not visibly influence the molecular architecture depicted by conventional electron microscopy of negatively stained samples. In this context, it is interesting to note that the H^+ -translocating F_1F_0 -ATPases from the homoacetogenic bacteria *Clostridium thermoautotrophicum* and *Clostridium thermoaceticum* appear to contain only 6 subunits [22]; nevertheless, electron micrographs of the F_1 -ATPases from the latter organism were indistinguishable, within the limits of resolution, from F_1F_0 -ATPases containing 8 subunits [16]. Further insight into the structural properties of the various F_1F_0 -ATPases and their functional implications can only be obtained by application of high resolution imaging techniques [23,24].

Acknowledgements: This work was supported by a grant from the Deutsche Forschungsgemeinschaft. The skilful technical assistance of S. Bowien is gratefully acknowledged. We are also indebted to Dr. R. Wilms for valuable advice.

References

- [1] Fuchs, G. (1986) FEMS Microbiol. Rev. 39, 181–213.
- [2] Heise, R., Müller, V. and Gottschalk, G. (1989) J. Bacteriol. 171, 5473–5478.
- [3] Yang, H. and Drake, H.L. (1990) Appl. Environ. Microbiol. 56, 81–86.
- [4] Geerligs, G., Schönheit, P. and Diekert, G. (1989) FEMS Microbiol. Lett. 57, 253–258.
- [5] Heise, R., Müller, V. and Gottschalk, G. (1993) FEMS Microbiol. Lett. 112, 261–268.
- [6] Heise, R., Reidlinger, J., Müller, V. and Gottschalk, G. (1991) FEBS Lett. 295, 119–122.
- [7] Reidlinger, J. and Müller, V. (1994) Eur. J. Biochem. 223, 275–283.
- [8] Laubinger, W. and Dimroth, P. (1988) Biochemistry 27, 7531–7537.
- [9] Laubinger, W. and Dimroth, P. (1989) Biochemistry 28, 7194–7198.
- [10] Kluge, C., Laubinger, W. and Dimroth, P. (1992) Biochem. Soc. Trans. 20, 572–577.
- [11] Penefsky, H.S. and Bruist, M.F. (1984) in: Methods of Enzymatic Analysis, vol. 4 (Bergmeyer, H.U., Ed.) pp. 324–335, Verlag Chemie, Weinheim.
- [12] Kresze, G.-B. (1983) in: Methods of Enzymatic Analysis, vol. 2 (Bergmeyer, H.U., Ed.) pp. 324–335, Verlag Chemie, Weinheim.
- [13] Tornqvist, H. and Belfrage, P. (1976) J. Lipid Res. 17, 542–545.
- [14] Kagawa, Y. and Racker, E. (1971) J. Biol. Chem. 246, 5477–5487.
- [15] Valentine, R.C., Shapiro, B.M. and Stadtman, E.R. (1968) Biochemistry 7, 2143–2152.
- [16] Mayer, F., Ivey, D.M. and Ljungdahl, L.G. (1986) J. Bacteriol. 166, 1128–1130.
- [17] Boekema, E.J., Schmidt, G., Gräber, P. and Berden, J.A. (1988) Z. Naturforsch. 43c, 219–225.
- [18] Pedersen, P.L. and Amzel, L.M. (1993) J. Biol. Chem. 268, 9937–9940.
- [19] Akey, C.W., Crepeau, R.H., Dunn, S.D., McCarty, R.E. and Edelstein, S.J. (1983) EMBO J. 2, 1409–1415.
- [20] Tiedge, H., Schäfer, G. and Mayer, F. (1983) Eur. J. Biochem. 132, 37–45.
- [21] Lünsdorf, H., Ehrig, K., Friedl, P. and Schairer, H.V. (1984) J. Mol. Biol. 173, 131–136.
- [22] Das, A. and Ljungdahl, L.G. (1993) FEBS Lett. 317, 17–21.
- [23] Harris, R. and Horne, R.W. (1994) Micron 25, 5–13.
- [24] Abrahams, J.P., Leslie, A.G.W., Lutter, R. and Walker, J.E. (1994) Nature 370, 621–628.