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IMMUNOLOGICAL AND FLUORESCENCE STUDIES WITH THE COUPLING FACTOR ATPase FROM *RHODOSPIRILLUM RUBRUM**

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SUMMARY

1. Purification of the coupling factor ATPase from *Rhodospirillum rubrum* has been achieved by a combination of a previously described procedure with chromatography on DEAE-Sephadex A50.

2. Identification of the coupling factor ATPase during purification, and estimation of the relative amount of the enzyme in each fraction was greatly simplified by utilization of its unusual fluorescence.

3. Preparations of *R. rubrum* coupling factor ATPase injected into rabbits yielded antisera which were suitable for following the course of purification.

4. Judged by immunoelectrophoretic analysis and polyacrylamide gel electrophoresis the final preparation was pure. Under standardized conditions, apparently pure preparations showed fluorescence ratios at 300/350 nm of 3–6, which are considerably higher than those reported for pure CF_1 from chloroplasts.

5. The enzyme lost its activity and changed its immunological identity during prolonged storage and by treatment with urea. Antisera against urea-treated enzyme showed the presence of two distinct antigens in the modified preparations.

INTRODUCTION

Immunological methods have been useful in the characterization of F_1 from beef heart mitochondria [1] and CF_1 from spinach chloroplasts [2]. Similar methods, used in the further purification and characterization of the previously described [3] coupling factor ATPase from *Rhodospirillum rubrum*, are presented in this paper.

When tested by polyacrylamide gel electrophoresis [3], the coupling factor ATPase from *R. rubrum*, purified according to the previously described procedure shows more than one band. Antisera against this preparation contained antibodies against up to 7 discernible antigens. Therefore, this preparation is not pure enough for a detailed study of the subunit structure of the enzyme, or for inducing monospecific antisera. Such antisera may be used for studying the role of the membrane-bound coupling factor ATPase, and especially for comparison with the reactions of inorganic pyrophosphate in chromatophores from *R. rubrum* [4, 5].

* Dedicated to Professor Dr W. Menke on the occasion of his 65th birthday.

More sensitive monitoring methods were needed during further purification of the enzyme. An assay method based on the unusual fluorescence properties of the coupling factor ATPase from *R. rubrum*, and analogous to that used for CF_1 from chloroplasts [6], is described. A further purification of the enzyme to apparent purity will be described in which chromatography on DEAE-Sephadex A50 is combined with the previously published procedure [3].

Dissociation of CF_1 from spinach has been achieved by treatment with urea and the products of dissociation were detected and characterized by immunological methods [7]. A similar characterization of products after urea treatment of the *R. rubrum* coupling factor ATPase will be described in this report.

MATERIALS AND METHODS

Reagents

All reagents were of analytical grade. The ATP was purchased from Boehringer, Mannheim GmbH (Mannheim, Germany), and agarose, nuclease (micrococcal) and ribonuclease-A (bovine pancreas) from Sigma. Glass-distilled de-ionized water was used for all solutions.

Preparation of chromatophores

Batch cultures of *R. rubrum* (van Niel Strain Sl) were grown as described previously [3, 8]. Chromatophores were prepared according to published procedures [5, 9], except for the method used for breaking the cells. 400–500 g wet weight of packed cells (equivalent to 50 l cell culture) were suspended in 0.2 M glycylglycine buffer, pH 7.4, to a total volume of 1.5 l, and then processed in a Sorvall-Ribi cell fractionator operated at 20 000 lb/in^2 and about 20 °C. DNAase and RNAase (approx. 2 mg each) were added to the resulting highly viscous suspension, after which it was set aside at room temp. for about 10 min. The resulting non-viscous suspension was centrifuged for 10 min at $9880 \times g$ (10 000 rev./min) in a Beckman Model J-21 centrifuge (JA-14 rotor). Chromatophores in the supernatant were sedimented by centrifugation for 2 h in a Beckman Model L2-65B ultracentrifuge using the Type 35 rotor at $70\,000 \times g$ (30 000 rev./min).

Purification of the coupling factor ATPase

The previously described purification method [3] was modified by introduction of chromatography on DEAE-Sephadex A50. Precycling of the ion exchanger was carried out as described by Lien and Racker [6]. The pooled fractions from the Sepharose 6B gel filtration step [3] were loaded onto the DEAE-Sephadex column (8×2.4 cm), which was equilibrated with 20 mM Tris · SO_4, 80 mM $(NH_4)_2SO_4$, pH 7.5. The column was washed with 100 ml of 20 mM Tris · SO_4, 160 mM $(NH_4)_2SO_4$, pH 7.5. The coupling factor ATPase was eluted by a linear gradient from 160 mM to 350 mM $(NH_4)_2SO_4$, 20 mM Tris · SO_4, pH 7.5, both solutions being 150 ml in volume. Those fractions showing highest fluorescence emission ratio at 300/350 nm were pooled and concentrated to 5 ml by ultrafiltration. The protein was precipitated with 55 % (w/v) $(NH_4)_2SO_4$ and allowed to stand on ice overnight. The precipitate was centrifuged and the pellet redissolved in a small volume (approx. 0.5 ml) of 50 mM Tris · HCl, pH 7.5 (plus 1 mM ATP). This sample was layered onto a sucrose

density gradient (8–25 %, w/v) and centrifuged in a Spinco SW40 rotor at 35 000 rev./min for 20 h. About 20–30 fractions were collected from the sucrose gradient.

All the purification steps were carried out at 0–4 °C.

Preparation of antisera

Immunization of rabbits was carried out as described previously for CF_1 [10]. Three rabbits (Nos B54, B55, B69) were primed each with 300 μg of a *R. rubrum* coupling factor ATPase preparation purified according to previously published procedures [3], and boostered with 250 μg of subsequent preparations. Three rabbits (Nos B56, B57, B70) were similarly treated twice with about 200 μg of a slightly impure preparation of the coupling factor ATPase which had been modified by treatment with 8 M urea for 30 min at 0 °C, and two rabbits (Nos B62, B63) were immunized twice with 200 μg of preparations of the enzyme that were pure by the criteria given in this paper. The sera were not dialyzed, but heated at 56 °C for 30 min to destroy the complement system, and stored at −28 °C.

Analytical methods

ATPase activity was measured by methods described previously [3, 11] and protein concentration was estimated by the Lowry method [12].

Polyacrylamide gel electrophoresis was performed by the procedure described by Davis [13]. After staining with Coomassie brilliant blue, the gels were scanned at 540 nm in a Pye Unicam Ultraviolet Spectrophotometer equipped with a gel-cuvette.

All fluorescence measurements were made at room temp. in an Aminco-Bowman spectrophotofluorimeter (Cat. No. 4-8911E), equipped with an Aminco Ratio Photometer (Cat. No. 4-8912). The RCA 1P21 photomultiplier tube was operated at 600 V. One mm slits were inserted into both the excitation (280 nm) and emission beams (slots 3 and 4). The excitation light was amplified by use of the standard planar mirror. The fractions from the Sepharose 6B and DEAE-Sephadex A50 columns were measured without dilution. 25 μl samples from the sucrose density gradient fractions were diluted with 1 ml of glass-distilled water.

Immunological tests

Ring tests, double diffusion in agarose and immunoelectrophoretic analysis with the LKB-Camag equipment were performed according to standard procedures [14]. Tests for agglutination of chromatophores were carried out on microscope slides by mixing small droplets of chromatophore suspensions (0.5 mg bacteriochlorophyll/ml in 0.15 M NaCl) and serum. The extent of agglutination was assessed by phase contrast microscopy at a magnification of ×500.

RESULTS AND DISCUSSION

Purification of the coupling factor ATPase from R. rubrum

Immunological studies during purification. Preparations of the coupling factor ATPase from *R. rubrum*, purified according to previously described methods [3] and injected into rabbits, yielded antisera with antibodies against more than one antigen. Immunoelectrophoretic analysis showed that up to 7 antigens could be distinguished (Fig. 1). The number of precipitation arcs was greater than the number of bands

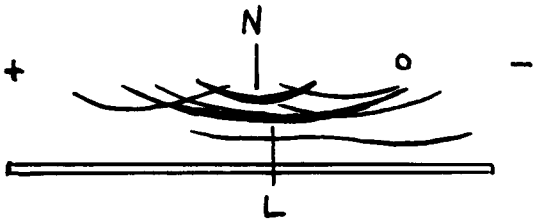

Fig. 1. Schematic drawing based on a combination of several immunoelectrophoretic runs of crude coupling factor ATPase from *R. rubrum* and antisera against the crude enzyme. N = precipitine arc formed with native enzyme; L = precipitine arc formed with degradation product.

TABLE I

FLUORESCENCE RATIO AND DEGREE OF PURIFICATION OF THE COUPLING FACTOR ATPase FROM *R. RUBRUM*

Purification steps: 1, crude extract; 2, 30–55 % ammonium sulfate fraction; 3, eluate from Sepharose 6B; 4, eluate from DEAE-Sephadex A50; 5, eluate from sucrose density gradient. Measurements were made on pooled fractions from each purification step. For assay of ATPase 480 μg protein from step 1 was used; 600 μg from step 2; 8 μg from step 3; 3 μg from step 4; 1.5 μg from step 5. The amount of Coomassie blue stain in the coupling factor ATPase was estimated by scanning the polyacrylamide gels, cutting out the peaks on the recorder paper, and weighing them. The peak corresponding to the coupling factor ATPase was identified by staining for ATPase [3]. The immunoelectrophoretic runs were performed with a serum against a half-crude coupling factor ATPase preparation, which contained antibodies against up to 7 discernible antigens. Fluorescence measurements were made as described in Materials and Methods. All fractions were measured at a protein concentration of 10 μg/ml.

Step	Total protein	Specific ATPase activity	Total ATPase activity	Stain in coupling factor ATPase	Arcs in immuno-electro-phoresis	Fluorescence ratio	
	(mg)	(μmol·min^{-1}·mg^{-1})	(μmol·min^{-1})	(% of total)	(number)	300/350	310/350
1	960	0.008	8	2	6–7	0.2	0.5
2	320	0.8	26	3.4	5–6	0.3	0.6
3	16	5.0	80	40	4	0.6*	0.9
4	3	10.7	32	85	2–3	2.6*	3.5
5	0.9	15.0	14	100	1	5.3*	7.8

* Up to 0.8 in step 3, 2.6 in step 4 and 6 in step 5.

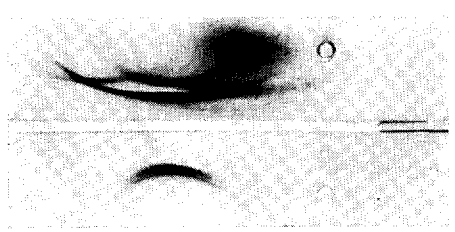

Fig. 2. Test for contaminating antigens in pure coupling factor ATPase from *R. rubrum*. Upper antigen well: 20 μg crude coupling factor ATPase after 30–55 % ammonium sulfate precipitation, containing antigen L; lower well: 2 μg coupling factor ATPase, purified according to the newly described method (after sucrose gradient); 50 μl antiserum B 69 in the trough.

usually seen after polyacrylamide gel electrophoresis of this preparation [3]. There were two major arcs, called N and L (Fig. 1). Usually all other antigens which were revealed by the antisera used could be removed from the partially purified preparation by chromatography on DEAE-Sephadex A50 after the Sepharose 6B step (Table I). In the final preparation after sucrose density gradient centrifugation, only arc N (formed with the native enzyme) was left (Fig. 2). The line splitting seen in Fig. 2 is explained below.

Identification of the coupling factor ATPase during purification by measuring its fluorescence. It was found that fractions showing the highest fluorescence emission ratio at 300/350 nm were those containing highest ATPase activity. As is evident from Fig. 3, this was the case for fractions obtained at different steps during purification and indicates that the unusually low fluorescence intensity at 350 nm is an intrinsic property of the ATPase. Consequently, the coupling factor ATPase from *R. rubrum* can readily be detected in fractions from chromatography columns and sucrose density gradients without the need for assaying ATPase or recoupling activity. The

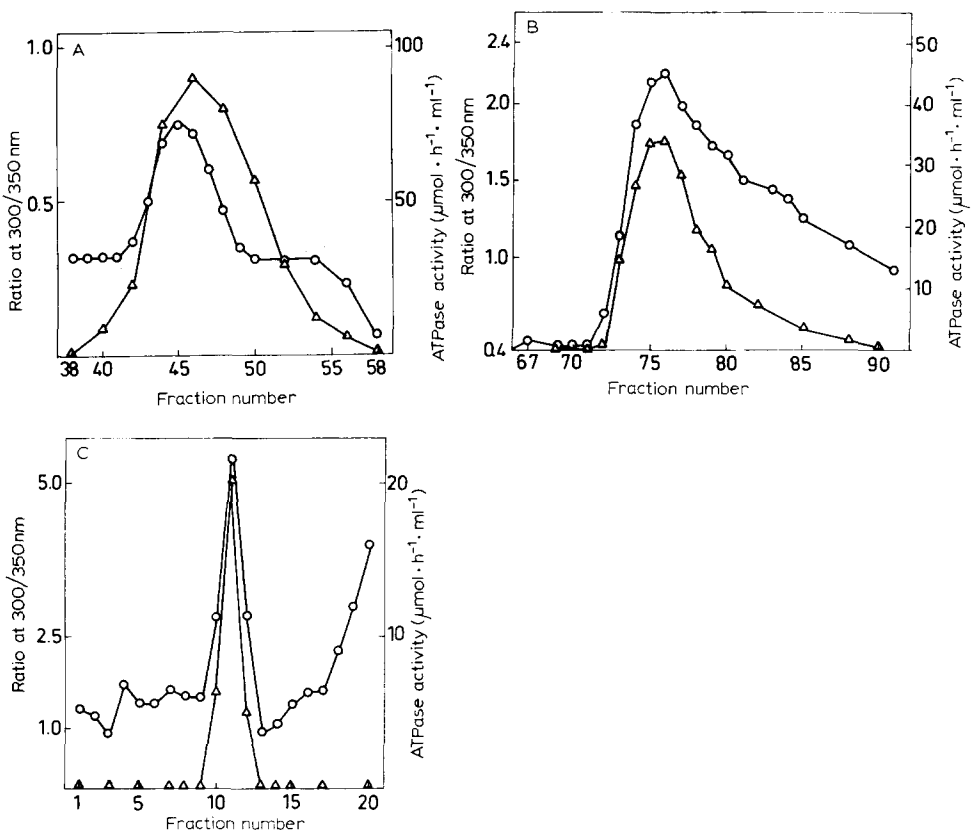

Fig. 3. Fluorescence ratio at 300/350 nm and ATPase activity in fractions from Sepharose 6B (a), DEAE-Sephadex A50 (b), and from the sucrose density gradient (c). Measurements were performed as described in Materials and Methods. 25 μl samples from the Sepharose-fractions, 50 μl samples from the DEAE-Sephadex-fractions and 5 μl samples from the sucrose gradient were used for the ATPase assay. ○–○, ratio at 300/350 nm; △–△, ATPase activity.

method is especially helpful in localizing the enzyme in fractions from the DEAE-Sephadex A50 column. In these fractions the protein concentration is very low.

Criteria for purity of the coupling factor ATPase. From the results of immunoelectrophoretic analysis with antisera against the coupling factor ATPase purified according to previous procedures [3] it follows that the contaminating proteins are able to induce an antibody response. A monospecific serum can be induced only if they are removed. After the final purification step described in this paper the preparation of the coupling factor ATPase was pure as judged from polyacrylamide gel electrophoresis (not shown). Indeed, antiserum B62 which was produced by injecting this preparation shows only one precipitation arc (N) if tested even with a crude preparation (Fig. 4). Within the limits of sensitivity of the methods this preparation of the coupling factor ATPase from *R. rubrum* therefore appears homogeneous.

Fluorescence properties of pure coupling factor ATPase. There is a correlation between the fluorescence emission ratio at 300/350 nm and the degree of purity of the coupling factor ATPase from *R. rubrum* (Table I). Pure preparations, as judged from polyacrylamide gel electrophoresis and from immunoelectrophoretic analysis (Fig. 2), show ratios between 3 and 6. Since most other proteins have a much lower 300/350 nm emission ratio than the coupling factor ATPase from *R. rubrum*, the determination of this ratio proved to be a rapid and convenient way of estimating the degree of purity of that enzyme. The relationship between the 300/350 nm emission ratio and the degree of purity is, however, not linear. Addition of bovine serum albumin to a purified preparation of the coupling factor ATPase resulted in a hyperbolic curve when the 300/350 nm emission ratio was plotted against the amount of bovine serum albumin added. Thus, the fluorescence method is very sensitive towards changes in the degree of purity when only traces of contaminating proteins are present, but relatively insensitive in crude preparations. Another limitation of the method is that a contaminating protein with a high tryptophan content will affect the ratio much more than a protein with low tryptophan.

CF_1 purified according to published procedures [6] and measured in our fluorimeter under the same conditions as the *R. rubrum* coupling factor ATPase, showed a 300/350 nm ratio of 2.14.

The sensitivity of our fluorimetric routine measurements is demonstrated by the data shown in Table II.

Whereas the fluorescence emission spectrum of both crude *R. rubrum* coupling factor ATPase and bovine serum albumin show an additional peak around 335 nm

Fig. 4. Test for specificity of antiserum B 62 against pure coupling factor ATPase from *R. rubrum*. Antigen well: 20 μg crude coupling factor ATPase (same as Fig. 2); upper trough, mixture of two antisera against crude coupling factor ATPase (5 μl serum B54 and 25 μl serum B69); lower trough, 50 μl serum B62.

TABLE II

SENSITIVITY OF THE FLUOROMETRIC METHOD

The samples from the DEAE-Sephadex A50 purification step were dissolved in 1 ml of water and measured as described in Materials and Methods. The instrument was set at maximal sensitivity.

RrF_1 (μg)	Contamination with bovine serum albumin (%)	300 nm/350 nm ratio	Change in 300/350 nm ratio (%)
0.5	0	2.3	–
	4	2.1	9
	8	1.75	24
1.0	0	2.5	–
	2	2.1	16
	4	1.85	26
10.0	0	2.6	–
	0.2	2.46	5
	0.4	2.34	10

(Fig. 5), the pure coupling factor ATPase shows only one peak around 310 nm. This is in good agreement with the published value of 310 ± 5 nm for tyrosine [15]. It can be assumed from the emission spectrum that the coupling factor ATPase from *R. rubrum* like F_1 [16] and CF_1 [17] lacks tryptophan.

In the initial stages of this work no fluorescence spectrum of the pure coupling factor ATPase from *R. rubrum* was available and thus when measuring fluorescence ratios we adopted the wavelengths used by Lien et al. for CF_1 [6]. These authors reported a fluorescence emission peak at 302 nm for pure CF_1.

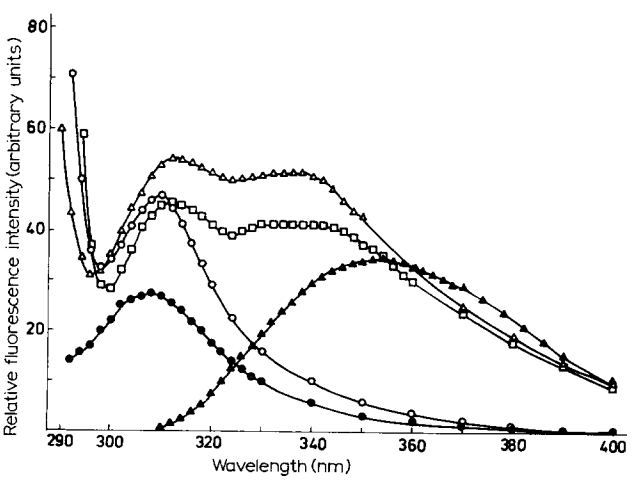

Fig. 5. Fluorescence emission spectra. Measurements were performed as described in Materials and Methods. ○–○, pure coupling factor ATPase from *R. rubrum* (10 μg); △–△, crude coupling factor ATPase (10 μg); □–□, bovine serum albumin (10 μg); ●–●, tyrosine; ▲–▲, tryptophan.

Studies on the degradation of the coupling factor ATPase from R. rubrum

Modification upon storage. A pronounced decrease in ATPase activity upon storage of the coupling factor ATPase from *R. rubrum* was accompanied by the appearance of a band in the anodic part of polyacrylamide gels after electrophoresis. The strength of this band increased with the time the sample had been stored. If immunoelectrophoretic analysis was performed with the coupling factor ATPase after one or two weeks of storage at 0 °C, the arc N, formed with the native enzyme, was diminished and L, the other major arc observed, was largely increased. Thus the product formed after storage seems to be a different antigen. In fact, analysis of fresh preparations often showed a splitting of the main precipitation arc (N) (Fig. 2). This was probably the result of a formation, during immunodiffusion and washing of the plates,of the product giving rise to arc L. Antiserum B62 did not contain antibodies against the antigen forming arc L and therefore, as shown in Fig. 4, the line splitting was not seen.

Modification by urea, KSCN or sodium dodecyl sulfate. Treatment with 3 M urea inactivated the ATPase within one hour at 0 °C (Table III) and the band in polyacrylamide gel electrophoresis corresponding to the native enzyme disappeared, concomitantly with a strengthening of a band corresponding to that obtained upon storage. A similar decrease of arc N and increase of arc L was seen in immunoelectrophoretic analysis of urea treated coupling factor ATPase (Fig. 6a and b). After 90 min in 3 M urea, arc N disappeared completely. Arc L was detected in partially purified fractions containing the coupling factor ATPase but the antigen forming this arc stayed in the top half of the sucrose gradient (L = arc formed with light antigen). Together with the higher migration in polyacrylamide gels this indicates a much lower molecular weight of the antigen giving rise to arc L as compared with the native enzyme.

Dissociation of chloroplast CF_1 into several products had been detected with antisera against the urea treated enzyme [7]. An immunization was therefore performed with the coupling factor ATPase from *R. rubrum* after urea treatment. The rabbits B56, B57 and B70 synthesized specific antibodies against the antigen forming arc L but the sera do not seem to contain antibodies against the native enzyme (Fig. 7a, b).

TABLE III

EFFECT OF UREA ON THE PURIFIED COUPLING FACTOR ATPase FROM *R. RUBRUM*

4 μg of protein, dissolved in water to a total volume of 0.3 ml, was incubated with urea for 1 h on ice, before assay of ATPase as described in Materials and Methods.

Urea (M)	Specific ATPase activity ($\mu mol \cdot min^{-1} \cdot mg^{-1}$)	Activity (% of control)
0	10	100
1	6	62
2	0.3	3
3	0	0

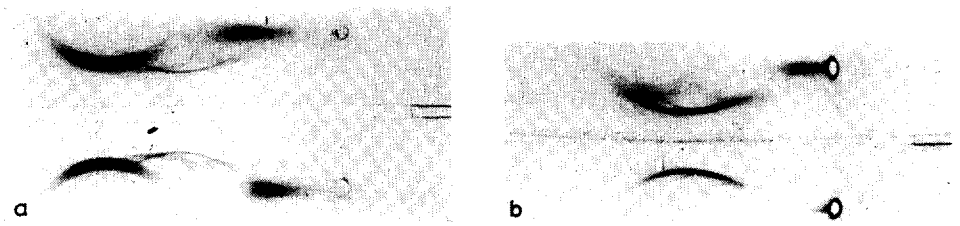

Fig. 6. Degradation of the coupling factor ATPase from *R. rubrum*. (a) upper well, 3 μg of coupling factor ATPase and contaminations (no urea treatment); lower antigen well, same antigen, treated with 1 M urea for 90 min at room temp. (b) upper well, same antigen, treated with 2 M urea; lower well, same antigen, treated with 3 M urea; troughs, 10 μl serum B54 and 40 μl 0.1 M NaCl. The gels contain 0.5 M urea which slows down the migration of the antigen forming arc L.

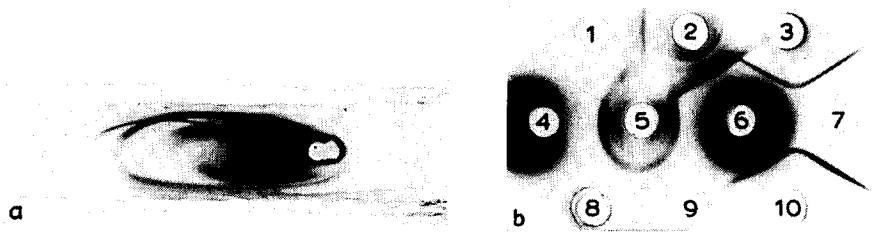

Fig. 7. (a) Test for antibodies contained in serum B56 against urea treated coupling factor ATPase from *R. rubrum*. Antigen well, 20 μg partially purified coupling factor ATPase (as Fig. 2); upper trough, mixture of two antisera against partially purified coupling factor ATPase (as Fig. 4); lower trough, 50 μl antiserum B56. The gel contains no urea. (b) Test for crossreactions and specificity of sera. Wells: 1, coupling factor ATPase from *R. rubrum* partially degraded by storage; 2, serum B56; 3, serum B62; 4, crude coupling factor ATPase (as Fig. 2); 5, serum B70; 6, as well 4; 7, purified coupling factor ATPase partially degraded by storage; 8, crude extract; 9, purified coupling factor ATPase degraded by 8 M urea; 10, serum B63. The gel contains 0.5 M urea.

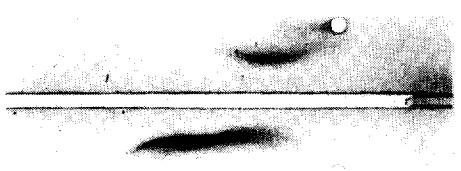

Fig. 8. Test for dissociation of the coupling factor ATPase from *R. rubrum* by urea and sodium dodecyl sulfate. Upper well, 2 μg purified coupling factor ATPase treated with 8 M urea (4 h at room temp.); lower well, same antigen, after 2 h treatment with 8 M urea plus 1 % sodium dodecyl sulfate; trough, 50 μl serum B70. The gel contains 0.5 M urea and 0.5 % Triton X-100, which considerably slows down the migration of all antigens.

Treatment of the enzyme with 8 M urea only gave rise to arc L as did treatment with 0.2 M KSCN. At concentrations of KSCN greater than 1 M also arc L disappeared. A combination of 8 M urea and 1 % sodium dodecyl sulfate (2 h at room temp.) yielded an antigen (arc L'), of much higher electrophoretic mobility, as upon immunoelectrophoresis with 0.5 M urea and 0.5 % Triton X-100 the antigen giving rise to arc L and the fast migrating antigen separated (Fig. 8).

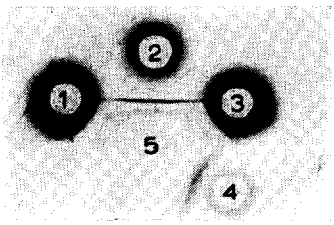

Fig. 9. Test for absorption of an antiserum against coupling factor ATPase from *R. rubrum* by chromatophores. Wells: 1, chromatophores from *R. rubrum*; 2, crude coupling factor ATPase (as Fig. 2); 3, as well 1; 4, degraded coupling factor ATPase; 5, antiserum B54.

Specificity of antisera against the coupling factor ATPase from R. rubrum

The antibodies against the native coupling factor ATPase do not react with the antigen forming arc L (Fig. 7b), and some antisera against the coupling factor ATPase do not even contain antibodies against this antigen (Fig. 4). Antisera against the modified enzyme do not react with the native coupling factor ATPase (Fig. 7). No antisera against the native coupling factor ATPase from *R. rubrum* precipitated CF_1 from chloroplasts, and conversely an antiserum against CF_1 (No. C 55-11) did not precipitate the coupling factor ATPase from *R. rubrum*. Antisera against CF_1 subunits α, β and δ [18] did not react with urea-treated *R. rubrum* coupling factor ATPase, but serum B56 and B70 showed a trace of antibody against urea-treated CF_1, although they did not precipitate native CF_1 (not shown). It could not be decided which dissociation product of CF_1 was precipitated. This cross-reaction with antiserum against the urea-treated coupling factor ATPase from *R. rubrum* and urea-treated CF_1 needs further exploration. The result shows that immunologically related subunits might be hidden within immunologically distinct coupling factors.

In double diffusion with an antiserum against the coupling factor ATPase, towards the coupling factor ATPase and chromatophores from *R. rubrum*, a line of identity is seen (Fig. 9). There is no spur of the white line of precipitated coupling factor ATPase behind the red line of precipitated chromatophores. This indicates that no antigenic determinants in the soluble coupling factor ATPase are hidden when the enzyme is bound to the membrane.

As expected, agglutination of chromatophores from *R. rubrum* by antisera against the coupling factor ATPase from this organism was positive, whereas chromatophore preparations from *Rhodopseudomonas spheroides* (Ga) or *Rhodopseudomonas capsulata* (WT) were not agglutinated at all by serum B62. The positive agglutination shows that the coupling factor ATPase is accessible to the large nonpermeant antibody molecule, which confirms that this enzyme is located on the outside of the chromatophores, which is directed towards the cytoplasm in vivo. Evidence for inversion of the membrane in chromatophores relative to whole cells was obtained from the changed direction of the light-induced proton movements [19]. In an electron microscopic investigation of negatively stained chromatophores from *R. rubrum* [20] the characteristic protruding "knobs", in other systems shown to constitute the ATPase [21, 22] have been described as located on the outside surface of the chromatophores. Studies with ferritin-labelled antibodies might finally confirm the apparent identity of the "knobs" in *R. rubrum* with the coupling factor ATPase. Analysis of *R. spheroides* chromatophores by small angle X-ray scattering shows a

large protein protruding from the chromatophore surface, which possibly is the coupling factor ATPase [23].

The ATPase activity of purified coupling factor from *R. rubrum* was completely inhibited by the antisera against the native enzyme. Further results of inhibition studies will be described elsewhere (Johansson, B. C., Berzborn, R. J. and Baltscheffsky, M., in preparation).

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REFERENCES

1 Fessenden, J. M. and Racker, E. (1966) J. Biol. Chem. 241, 2483–2489
2 McCarty, R. E. and Racker, E. (1966) Brookhaven Symp. Biol. 19, 202–214
3 Johansson, B. C., Baltscheffsky, M., Baltscheffsky, H., Baccarini-Melandri, A. and Melandri, B. A. (1973) Eur. J. Biochem. 40, 109–117
4 Baltscheffsky, H., von Stedingk, L.-V., Heldt, H.-W. and Klingenberg, M. (1966) Science 153, 1120–1122
5 Baltscheffsky, M. (1967) Nature 216, 241–243
6 Lien, S. and Racker, E. (1971) Methods Enzymol. 23, 547–555
7 Lien, S., Berzborn, R. J. and Racker, E. (1972) J. Biol. Chem. 247, 3520–3524
8 Bose, S. K., Gest, H. and Ormerod, J. G. (1961) J. Biol. Chem. 236, PC13–14
9 Baltscheffsky, H. (1960) Biochim. Biophys. Acta 40, 1–8
10 Berzborn, R. J., Kopp, F. and Mühlethaler, K. (1974) Z. Naturforsch. 29c, 694–699
11 Taussky, H. and Shorr, E. (1953) J. Biol. Chem. 202, 675–685
12 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. I. (1951) J. Biol. Chem. 193, 265–275
13 Davis, B. J. (1964) Ann. N.Y. Acad. Sci. 121, 404–427
14 Campbell, D. H., Garvey, J. S., Cramer, N. E. and Sussdorf, D. H. (1970) Methods in Immunology, W. A. Benjamin, New York
15 Duggan, D. E., Bowman, R. L., Brodie, B. B. and Udenfriend, S. (1957) Arch. Biochem. Biophys. 68, 1–14
16 Penefsky, H. S. and Warner, R. C. (1965) J. Biol. Chem. 240, 4694–4702
17 Farron, F. (1970) Biochemistry 9, 3823–3828
18 Berzborn, R. J. (1972) Hoppe-Seyler's Z. Physiol. Chem. 353, 693
19 von Stedingk, L.-V. (1969) Progress in Photosynthesis Research (Metzner, H., ed.), Vol. III, pp. 1410–1419, Tübingen
20 Löw, H. and Afzelius, B. A. (1965) Exp. Cell Res. 35, 431–434
21 Racker, E. and Horstman, L. L. (1967) J. Biol. Chem. 242, 2547–2551
22 Lien, S. and Racker, E. (1971) J. Biol. Chem. 246, 4298–4307
23 Pape, E. H., Menke, W., Weick, D. and Hosemann, R. (1974) Biophys. J. 14, 221–232