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Phosphorylation and dephosphorylation reactions in chromatophores of *Chromatium vinosum* and *Chromatium tepidum*

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Phosphorylation and dephosphorylation reactions were studied in situ by ^{31}P-NMR on chromatophores from purple sulfur bacteria (*Chromatium (C.) vinosum*, and *Chromatium tepidum*) with special emphasis on examination of adenylate kinase as well as of H^+-ATPase and of their thermal natures. The phosphorylation reactions under light conditions with CCCP (carbonyl cyanide *m*-chlorophenylhydrazone) showed a reaction profile to produce ATP and AMP simultaneously in equal amounts which is similar to that under dark conditions. This suggested the existence of an adenylate kinase which catalyzes the reaction ADP → ATP + AMP without membrane potential. Activity after purification of the chromatophore with several washes or by sucrose density gradient centrifugation suggested that some adenylate kinases exist membrane-bound. The effect of temperature on photophosphorylation reactions in chromatophores obtained from *C. vinosum* and *C. tepidum* revealed that the H^+-ATPases kept their activity up to 50 and 65 °C, respectively, for *C. vinosum* and *C. tepidum*, which are well above the growing temperature limits. It was also suggested that the coupling factors of the H^+-ATPases (F_1) were cleaved from the membrane by the heat treatment at around 60 and 70 °C, respectively, for *C. vinosum* and *C. tepidum*, and that these coupling factors had ATP hydrolysis activity up to 70–80 and 100 °C, respectively, for *C. vinosum* and *C. tepidum*. Furthermore, adenylate kinases were found stable against heat treatment at up to 90 and 100 °C, respectively, for *C. vinosum* and *C. tepidum*.

Introduction

In a previous paper [1] photophosphorylation and dephosphorylation reactions were studied in situ by ^{31}P-NMR on chromatophores from the purple sulfur bacterium *Chromatium* (*C.*) *vinosum*. The paper demonstrated the capability of ^{31}P-NMR to pursue time-courses of the phosphorylation reaction in real time. It also showed that phosphorylation and dephosphorylation reactions resulted in production and consumption of AMP in addition to the H^+-ATPase reaction which interconverts ATP, and ADP and inorganic phosphate (P_i). It is known that adenylate kinase, which catalyzes the reaction from ADP to ATP and AMP, is present in chloroplasts [2,3] and photosynthetic bacteria [4,5]. Both soluble and membrane-bound adenylate kinases have been found in higher plants and photosynthetic bacteria. In this paper we characterize the enzymes which participate in phosphorylation and de-

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; Hepes, 4(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 2(*N*-morpholino)ethanesulfonic acid; PCr, sodium creatine phosphate.

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Data supplementary to this article are deposited with, and can be obtained from: Elsevier Science Publishers B.V., BBA Data Deposition, P.O. Box 1345, 1000 BH Amsterdam, The Netherlands. Reference should be made to BBA/DD/483/1060 (1991) 189. The supplementary information includes: Time-course of relative intensities of α-phosphorus signals of adenylates in the presence of myokinase (yeast), and of nucleotides in the chromatophore suspension (which was purified by sucrose density-gradient centrifugation) under illumination.

phosphorylation reactions in chromatophores of purple sulfur bacteria by comparison of the reactions occurring in the light and in darkness in the presence or absence of CCCP (carbonyl cyanide *m*-chlorophenylhydrazone) by utilizing a ^{31}P-NMR method. Chromatophore membranes were washed or were subjected to sucrose density gradient centrifugation to characterize the enzyme as to its cellular location. Effects of heat treatment on the reactions were also explored on chromatophores from both a mesophilic bacterium (*C. vinosum*) and a thermophilic bacterium (*C. tepidum*).

Materials and Methods

Materials

AMP, ADP, ATP, PCr (creatine phosphate) and myokinase (yeast) were purchased from Oriental Yeast Co. Ltd., and CCCP was obtained from Sigma. Hepes and Mes were from Dojin Chemicals and other reagents were guaranteed grade purchased from Wako Chemicals. Cultivation methods were previously described in Refs. 6, 7 and 8, 9 for *C. vinosum* and *C. tepidum*, respectively. Chromatophores were prepared by the grinding method with alumina powder as described previously [1]. The ground mixture was suspended in buffer A (400 mM KH_2PO_4 2 mM EDTA/5 mM $MgSO_4$ at pH 7.0). The suspension was centrifuged at 5000 × *g* for 5 min to remove the alumina powder and the supernatant was centrifuged again at 15 000 × *g* for 15 min in order to remove the debris. The crude chromatophore preparation was collected by high-speed centrifugation (100 000 × *g*, 80 min). The preparation was washed three times in Buffer B (100 mM KCl/25 mM glycylglycine/25 mM Mes/25 mM Hepes/5 mM $MgSO_4$/5 mM sodium succinate) and collected by high-speed centrifugation.

Methods

The measurements of ^{31}P-NMR at 121.48 MHz were performed on a Bruker CXP-300 FT NMR spectrometer equipped with a broad band (BB) probe which covers a 30–125 MHz range as described previously [1]. The observations were carried out under internal ^{2}H-lock using D_2O with 10-mm NMR tubes. Light illumination on chromatophore suspensions was achieved by employing a lighting system previously described [1]. A fiber-optical cable fixed in an adapter was inserted in a sample tube which was spun at 30–40 rpm at 25 °C. The sample was illuminated with a tungsten-halogen lamp whose light power was adjusted by changing the distance between lamp and optical-fiber terminal. Chromatophores were resuspended in 2 ml of buffer B and 0.5 ml of D_2O with 2 mM of sodium creatine phosphate (as an internal standard), and 20 mM KH_2PO_4 and 20 mM ADP were added, and the external pH was adjusted with 0.1 M KOH or 0.1 M HCl. Final sample volume was 3 ml and absorbance at 800 nm (A_{800}) = 50. ^{31}P-NMR signals were accumulated 250 times with a 30 ° pulse and 0.5 s recycling time in the first 10 min, and after 10 min the accumulation was conducted 500 times with the same setting to improve signal to noise ratios.

Absorption spectra were recorded on a recording spectrophotometer, UVIDEC-510 (Jasco) or UV-365 (Shimadzu). CD spectra were obtained on a recording circular dichrometer Jasco J-500 C with a data processor DP-500 (Jasco) as described previously [10].

Protein concentration was determined according to the method described by Lowry et al. [11]. BChl *a* concentration was estimated from A_{800} of the sample using the in vivo millimolar extinction coefficient of BChl *a* given by Clayton [12].

Sucrose density gradient centrifugation for purification of chromatophores was performed in a discontinuous gradient containing 5 ml each of 60, 50, 40, 30, 20 and 10% sucrose in buffer solutions. The chromatophore solution was layered on the top of the gradient and centrifuged at 100 000 × *g* for 90 min. The fraction partitioning in the 30–20% region was collected and used after washing with buffer B.

The effect of temperature on the reaction was examined after treatment of the chromatophore suspensions at specific temperatures for 30 min. After heat treatment the chromatophores were cooled to room temperature and the reaction was observed at 25 °C as described above.

Results

Adenylate kinase in C. vinosum

Fig. 1 shows a time-course of a phosphorylation reaction in chromatophores from *C. vinosum*. The rela-

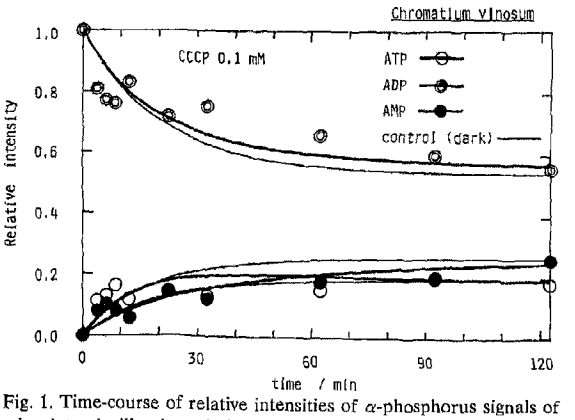

Fig. 1. Time-course of relative intensities of α-phosphorus signals of adenylates in illuminated chromatophore suspensions with 0.1 mM CCCP. The adenylate concentrations in equilibrium were estimated by ^{31}P-NMR. Concentration: A_{800} = 50, 6.5 mg protein/ml, BChl *a* concentration = 0.45 mg ml^{-1}; initial external pH: 7.2–7.3. Light intensity = 86 mW.

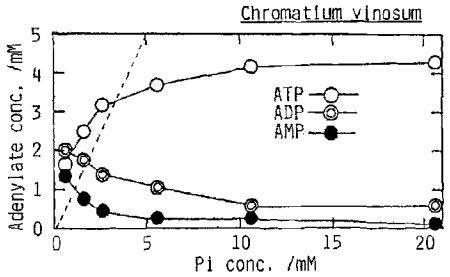

Fig. 2. The concentration dependence of photophosphorylation on inorganic phosphate (KH_2PO_4) in chromatophores. The adenylate concentrations in equilibrium were estimated by ^{31}P-NMR. Conditions as for Fig. 1.

tive intensity in the signal represents the ratio of the α-^{31}P-NMR magnitude of each phosphoadenylate divided by the total α-^{31}P-NMR magnitudes from all the phosphoadenylates present (ATP, ADP and AMP). This quantity corresponds to the relative concentration of each phosphoadenylate. The reaction was explored both under light conditions with CCCP, and under dark conditions. It is noted that the phosphorylation time courses in both conditions resemble each other and that equal amounts of both ATP and AMP were produced at the expense of ADP. Because there is no protonmotive force either in the light with CCCP or in the dark, H^+-ATPase cannot produce ATP from ADP and P_i under these conditions. The simultaneous formation of ATP and AMP from ADP implies the presence of a reaction catalyzed by an adenylate kinase.

Dependence of the phosphorylation and dephosphorylation reactions on concentrations of inorganic phosphate was examined when the reactions started from an ADP initial concentration of 5 mM (Fig. 2). The result shows that at concentration under 3 mM of P_i the produced ATP exceeded the amount of P_i added. This result cannot be explained by the reaction catalyzed only by H^+-ATPase, and implies that some phosphorylation or dephosphorylation independent of the P_i concentration occurs in the reaction, indicating the existence of an adenylate kinase.

The time-course of the reaction catalyzed by myokinase (a well-established soluble-type adenylate kinase [12,13]) resembled the one shown in Fig. 1, which provided additional evidence for the presence of an adenylate kinase (supplementary data deposited in BBA).

Fig. 3 shows the effects of wash of the chromatophores on the phosphorylation reaction. As described in the experimental section, the chromatophores used for the experiment were previously washed by buffer B three times to decrease the phosphate ion concentration outside the chromatophore vesicles. Further washes for the prepared chromatophores did not significantly remove the activity, which indicates that some

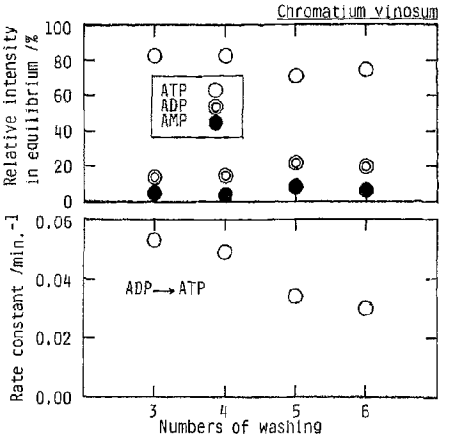

Fig. 3. The dependence of photophosphorylation activity in chromatophores on the number of wash steps. The adenylate concentrations in equilibrium were estimated by ^{31}P-NMR. Conditions as for Fig. 1.

adenylate kinases are present in the membrane of cells of *C. vinosum*. This is further strengthened by the fact that purification of the prepared chromatophore by sucrose density gradient centrifugation did not essentially remove the adenylate kinase activity as compared with the chromatophore before purification by density gradient centrifugation (supplementary data deposited in BBA).

Temperature dependence

Fig. 4 shows the temperature dependence of the phosphorylation reaction in the chromatophores from *C. vinosum* under light conditions. The rate for the

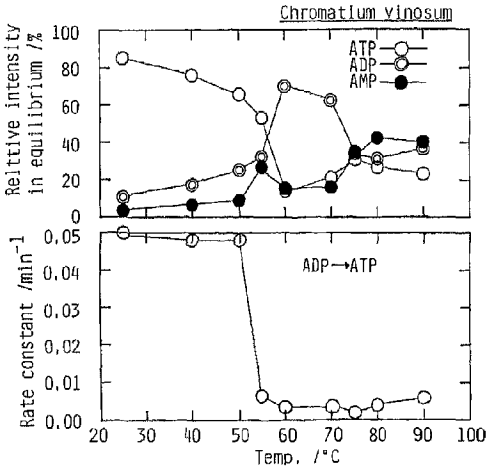

Fig. 4. Temperature-dependence of photophosphorylation in chromatophores from *C. vinosum*. The rate constant from ADP to ATP (bottom) and the relative intensities in equilibrium (top) were estimated by ^{31}P-NMR. Conditions as for Fig. 1.

photophosphorylation reaction from ADP to ATP is relatively constant up to 50 °C; above this temperature the rate dropped drastically (Fig. 4). However, even after heat treatment to 90 °C, the rate did not reach 0. The relative intensity of ATP in equilibrium decreased gradually above 30 °C, dropped sharply to a low level above 55 °C, and remained at about 20–30% above this temperature. Both the rate constant and the relative intensity in equilibrium showed a clear decrease of photophosphorylation activity around 55 °C. This may imply either a decrease in membrane potential or a decrease in the H^+-ATPase activity itself. The high equilibrium concentration of ADP and low level of ATP between 60 and 70 °C suggests that reaction increases the relative concentration of ADP at the expense of ATP. This could be attributed the activity of the coupling factor F_1 of H^+-ATPase. The reaction above 75 °C implies an adenylate kinase activity from the high concentration of AMP in equilibrium.

Fig. 5 demonstrates a control experiment for Fig. 4 under dark conditions. The rate of conversion of ADP to ATP shows a relatively constant value over the whole temperature range of the heat treatment. The reaction may be ascribed to an adenylate kinase activity. The relative intensity in equilibrium shows a relatively high ADP level at around 70–80 °C. This may imply the F_1 activity which catalyzes an ATP hydrolysis to ADP. It is noted that the reaction due to an adenylate kinase showed fairly high thermal stability against 30 min heat treatment at 90 °C.

Fig. 6 shows absorption and CD spectra of chromatophores from *C. vinosum* after heat treatment at different temperatures. The near infrared spectra between 700 at 1000 nm corresponds to the Q_y transi-

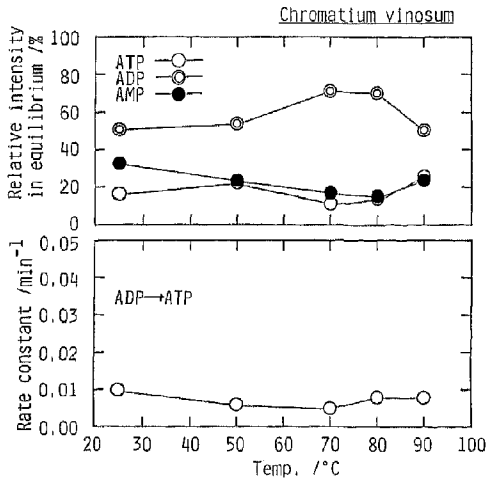

Fig. 5. Temperature-dependence of phosphorylation in chromatophores from *C. vinosum* under dark conditions. Conditions are the same as those in Fig. 1 except for the illumination condition.

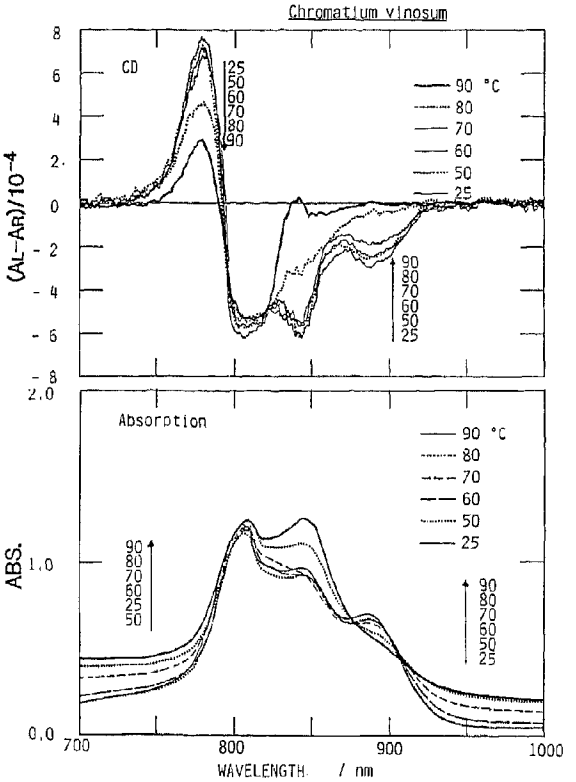

Fig. 6. The near-infrared absorption (bottom) and circular dichroism (top) spectra of chromatophores from *C. vinosum* in buffer B. The original sample, which was heated and used for ^{31}P-NMR measurements (Figs. 4, 5) was diluted 40-times with buffer B for measurement. The absorption spectrum was expressed by absorbance for the optical length of 1 cm and the circular dichroism spectrum magnitude is the difference of absorbance between the left and right circularly polarized lights.

tions of BChl *a* mainly from antennas. The present cultural condition yields *C. vinosum* with antenna absorbing at 810, 850 and 890 nm and had CD extrema at 775, 810, 850 and 890 nm [7]. Although changes of these peaks do not directly reflect the state of the adenylate transformation apparatus, they represent at least changes of the organization of antenna pigment-proteins in the membranes and a denaturation of the antenna pigment-proteins themselves. Gradual changes at temperatures higher than 50 °C in the CD around 775, 850 and 900 nm may indicate a modification of the organization of the B800-850 and B890 complexes in the membranes. Above 80 °C, the drastic changes in CD around 775, 850 and 900 nm indicate the collapse of the native structure of the B800-850 complexes in addition to that of the B890 complex. Comparison of the temperatures which induced the antenna absorption and CD changes with those for the phosphorylation activity implies that chromatophores lose photo-

phosphorylation activity in accordance with the minor CD change, which may suggest a modification of the organization of the antenna pigment-proteins.

To compare the effect of temperature on the phosphorylation reaction of the mesophilic purple sulfur photosynthetic bacterium (*C. vinosum*) with that for a thermophilic one, the phosphorylation reactions were observed for *C. tepidum* and the results are shown in Fig. 7. The rate constant for photophosphorylation of ADP to ATP stayed at a fairly high level up to 50 °C, but above this temperature it decreased to a medium level and stayed at this level up to 65 °C, then further decreased at higher temperatures. It should be noted that the rate of photophosphorylation in *C. tepidum* membranes remained at about 20% of the maximum level, even after 30 min heat treatment at 100 °C. The relative concentration of ATP in equilibrium remained constant value up to 65 °C, then suddenly decreased to almost 0 at 70 °C, and above that temperature it settled at about 10%. A clear change around 70 °C was observed in the results both for the rate constant and the relative intensity in equilibrium. The high equilibrium concentration of ADP, and low concentration of ATP above 70 °C was ascribed to the F_1 activity hydrolyzing ATP to ADP and P_i. The reaction in the higher temperature range (above 75 °C) reflects adenylate kinase activity and F_1-ATP hydrolyze activity, because of the similar equilibrium concentration of AMP relative to that of ATP, and the high ADP concentration. The relatively high ADP to ATP reaction rate, which remained up to 100 °C, was very noticeable for the thermophilic bacterium and indicated the high thermostability of the adenylate kinase and the coupling factor F_1.

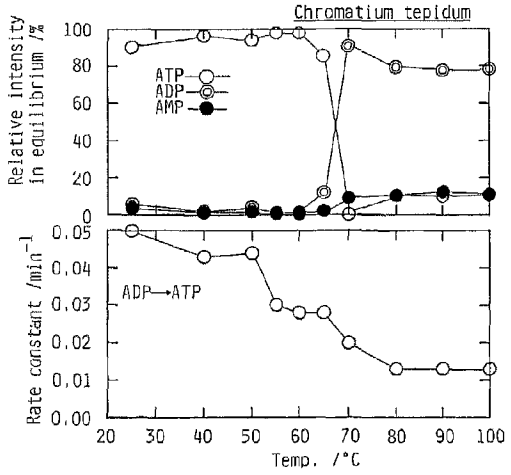

Fig. 7. Temperature-dependence of photophosphorylation reaction in chromatophores from *C. tepidum*. The rate constant from ADP to ATP (bottom) and the relative intensities in equilibrium (top) were estimated by ^{31}P-NMR. Conditions as for Fig. 1.

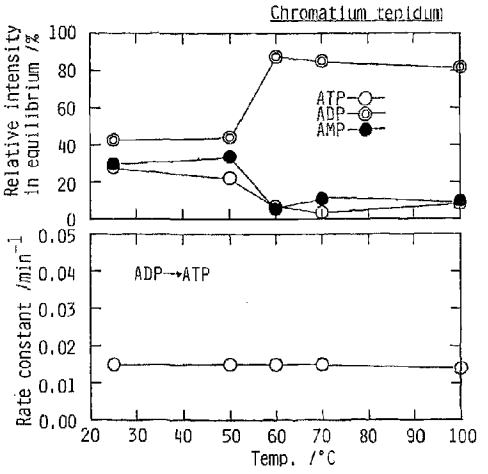

Fig. 8. Temperature-dependence of phosphorylation in chromatophores from *C. tepidum* under dark conditions. Conditions are the same as those in Fig. 7 except for the illumination condition.

Fig. 8 demonstrates a control experiment for Fig. 7 under dark conditions. The rate from ADP to ATP shows a relatively constant value over the whole temperature range for heat treatments. The relative intensity in equilibrium shows a relatively high ADP level, especially above 60 °C. Comparison of the results under both light and dark conditions demonstrates that a similar reaction happens under both light and dark conditions at temperatures above 70 °C. This suggests an adenylate kinase reaction and the F_1 activity which show high temperature stability – as high as 30 min heat treatment at 100 °C.

Fig. 9 shows absorption and CD spectra after heat treatment at different temperatures. Minor changes in the CD bands around 775, 800, 850 and 920 nm indicate a modification of B800-850 and B920 up to 70 °C and sharp changes in the CD bands around 775, 800, 850 and 920 nm above 80 °C indicate destruction of the intrinsic structure of the B800-850 as well as B920 above 80 °C. Comparison of the temperatures which induced the antenna absorption and CD changes with those for the phosphorylation activity implies that chromatophores lose photophosphorylation activity in accordance with the minor CD change, which may suggest a modification of the organization of the antenna pigment-proteins.

Discussion

Presence of adenylate kinase

In a previous paper we have demonstrated the existence of an enzyme which regulates AMP concentration in chromatophores of purple sulfur bacteria (*C. vinosum*) in addition to the H^+-ATPase which adjusts the concentration of ATP and ADP. Whether this

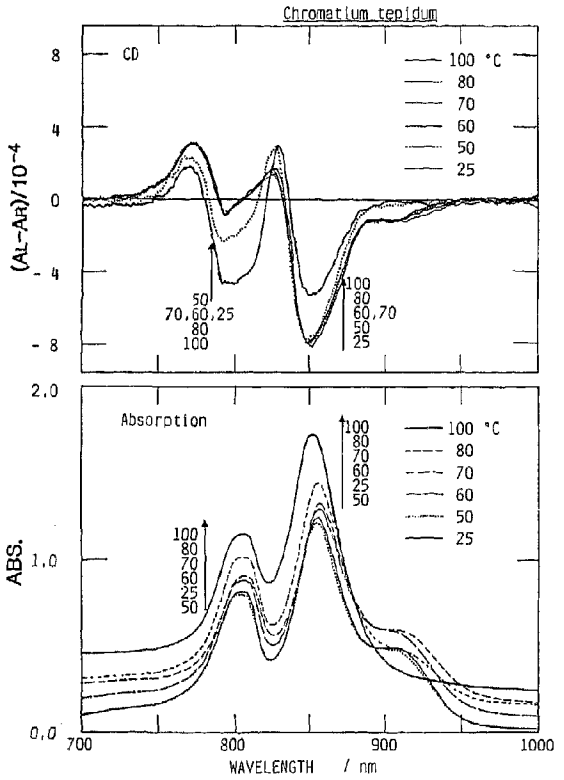

Fig. 9. The near-infrared absorption (bottom) and circular dichroism (top) spectra of chromatophores from *C. tepidum* in buffer B. The original sample which was heated and used for ^{31}P-NMR measurements (Figs. 7, 8), was diluted 40-times with buffer B for measurement. The absorption spectrum was expressed by absorbance for the optical length of 1 cm and the circular dichroism spectrum magnitude is the difference of absorbance between the left and right circularly polarized lights.

AMP-related reaction undergoes light-induced photophosphorylation (adenylate + $P_i \rightarrow$ AMP) or a light independent one has not been well examined. The uncoupler CCCP dissipates the proton concentration gradient across the membrane in chromatophores and thus inhibits photophosphorylation. Fig. 1 shows that, in the presence of 0.1 mM of CCCP, the phosphorylation reaction time-course in the light resembled that in the dark; both light with CCCP and dark reactions produced AMP and ATP simultaneously in equal amounts. This result implies that the AMP-related reaction is independent of the proton membrane potential, thus differing from the photophosphorylation reaction. The concentration dependence of the phosphorylation reaction on P_i (Fig. 2) has shown that the reaction independent of P_i proceeds at a relatively low concentration of P_i These two observations suggested the existence of an adenylate kinase which catalyzes the reaction

$$2ADP \rightleftarrows AMP + ATP \quad (1)$$

Fig. 10 shows the plot of adenylate levels against the Atkinson's Energy Charge value (E.C.) [16,17] from the experimental results shown in Fig. 2. The E.C. value can be described in the following equation

$$E.C. = \frac{0.5[ADP] + [ATP]}{[AMP] + [ADP] + [ATP]} \quad (2)$$

The solid lines in Fig. 10 represent simulation curves for the Energy Charge value assuming the equilibrium constant, K, expressed in the Eqn. 3 to be 0.59 and presuming the total adenylate concentration to be constant (the presumption of Eqn. 4) which has been verified in the previous paper [1]).

$$K = \frac{[ATP][AMP]}{[ADP]^2} \quad (3)$$

$$[AMP] + [ADP] + [ATP] = \text{constant} \quad (4)$$

A very good fit between the experimental points and the simulation curves supplies further evidence for the presence of adenylate kinase.

The existence of adenylate kinase has been confirmed in chloroplasts [2,3], while in photosynthetic bacteria (*R. palustris*, *R. sphaeroides* and *R. rubrum* [4,5]) soluble adenylate kinases have been found. A membrane-bound adenylate kinase in chromatophores has been found recently for *R. sphaeroides* by Koyama et al. [15]. Müller et al. reported that in *R. palustris*, *R. sphaeroides* and *R. rubrum* washing more than three times completely removed adenylate kinase activity. In

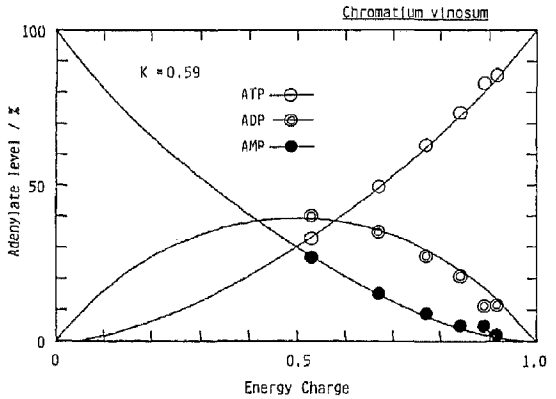

Fig. 10. The plot of adenylate composition against Energy Charge values. Circles: experimental values from Fig. 2. Solid lines: simulation curves for the equilibrium constant, K, of 0.59.

the present work the chromatophores obtained from *Chromatium vinosum* were washed three times but retained most of their enzymatic activity, and purification by a sucrose density gradient cut the enzymatic activity by only about one half. The activity clearly remaining after washing several times (Fig. 3) and after sucrose density gradient purification (figures deposited in BBA data deposition) is strong evidence that membrane-bound adenylate kinases exist in *C. vinosum*.

Thermostability of H^+-ATPase and adenylate kinase

The results in Figs. 4 and 7 demonstrated that H^+-ATPases in *C. vinosum* and *C. tepidum* have considerable thermal stability in vitro up to the temperature of their highest temperature of survival in nature. The H^+-ATPase derived from the thermophilic bacterium had a higher temperature stability than that from the mesophilic one. The difference in the maximum temperature required to maintain H^+-ATPase activity between *C. vinosum* (around 50 °C), and *C. tepidum* (65 °C) was essentially equal to that in their optimum growth temperatures, that is 25–30 °C for *C. vinosum*, and 45–50 °C for *C. tepidum*.

The reduction in H^+-ATPase activity may imply either the decrease of the membrane potential or the decrease of the H^+-ATPase activity itself. After the loss of H^+-ATPase activity the relative intensity of ADP in equilibrium increased at temperatures of 60–70 °C for *C. vinosum* and 70–100 °C for *C. tepidum*. Since the coupling factor F_1 of H^+-ATPase facilitates the increase of ADP by the hydrolysis of ATP by inverting the flow of the proton, or since the F_1 parted from F_0 catalyzes ATP hydrolysis, it is possible to explain the results by the F_1 activity. In the control dark reactions where both H^+-ATPase and adenylate kinase should be active under the temperature of 55 °C for *C. vinosum*, and 65 °C for *C. tepidum*, the ADP levels were not so high as at higher temperatures. Therefore, the hydrolysis activity of H^+-ATPase is not so high as that for the free F_1. Thus, at the temperature above 60 °C for *C. vinosum*, and 70 °C for *C. tepidum*, the reaction should be explained by the activity of the free coupling factor F_1. Hence, the temperature change experiment shows that the F_1 is cleaved from the chromatophore during the heat treatment.

Comparisons of the temperatures which induced antenna CD changes with those of the phosphorylation activity (Fig. 4) imply that the H^+-ATPase activity was lost (at 55 °C), in accord with rather minor CD changes which may represent a modification of the organization in the antenna pigment-proteins in the membrane. Judged from the extent of the changes in the CD and the photophosphorylation activity, the loss of the photophosphorylation activity may derive first from the cleavage of the F_1 factor rather than from the more drastic membrane break. The temperature range where the equilibrium ADP level is high, i.e., the thermostability of the free coupling factor F_1 was up to 70–80 °C for *C. vinosum*, and to 100 °C for *C. tepidum*.

Simultaneous production of AMP and ATP in similar amounts at temperatures as high as 90–100 °C indicates that the adenylate kinases from Chromatiaceae are extremely thermally stable. In general, adenylate kinases are known to be very thermostable [2–5]. The specific reasons for this for Chromatiaceae is not clear from the present experiment. Structural research after purification will be needed for this purpose.

References

1 Ohta, M., Nozawa, T., Hatano, M., Hayashi, H., Tasumi, M. and Shimada, K. (1989) Biochim. Biophys. Acta 1011, 33–39.
2 Murakami, S. and Strotmann, H. (1983) Arch. Biochem. Biophys. 185, 30–38.
3 Giersch, C. Huber, U., Kobayashi, Y., Inoue, Y., Shibata, K. and Heldt, H.W. (1980) Biochim. Biophys. Acta 590, 59–73.
4 Müller, H., Neufang, H. and Knobloch, K. (1983) Photobiochem. Phtobiophys. 6, 317–327.
5 Neufang, H., Müller, H. and Knobloch, K. (1983) Arch. Microbiol. 134, 153–157.
6 Hayashi, H. and Morita, S. (1980) J. Biochem. 88, 1252–1258.
7 Hayashi, H., Nozawa, T., Hatano, M. and Morita, S. (1982) J. Biochem. 91, 1029–1038.
8 Madigan, M.T. (1984) Science 225, 313–315.
9 Madigan, M.T. (1986) Int. J. Syst. Bacteriol. 36, 222–227.
10 Nozawa, T., Fukada, T., Hatano, M. and Madigan, M.T. (1986) Biochim. Biophys. Acta 852, 191–197.
11 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
12 Clayton, R.K. (1963) Biochim. Biophys. Acta 75, 312–323.
13 Kuby, S.A., Palmieri, R.H., Frischat, A. Fischer, A.H., Wu, L.H., Maland, L. and Manship, M. (1984) Biochemistry 23, 2393–2399.
14 Fry, D.C., Kuby, S. and Mildvan, A.S. (1986) Proc. Natl. Acad. Sci. USA 83, 907–911.
15 Koyama, Y, Nakano, T., Utsumi, H. and Yamamoto, S. (1989) Photobiochem. Photobiophys. 14, 227–236.
16 Atkinson, D.E. (1967) J. Biol. Chem. 242, 3239–3241.
17 Atkinson, D.E. (1968) Biochemistry 7, 4031–4034.