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Biochemistry

Specialized transducing phage λ carrying the genes for coupling factor of oxidative phosphorylation of *Escherichia coli*: Increased synthesis of coupling factor on induction of prophage λ*asn*

(coupling factor F_1–F_0/transducing phage/ATPase)

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ABSTRACT **Studies were made of the synthesis of the coupling factor complex (F_1–F_0) of oxidative phosphorylation after prophage induction of a set of *Escherichia coli* strains lysogenic for defective transducing phage λ*asn*, λ*uncA*, or λ*bglC*. The transducing phages had been isolated from a strain of *E. coli* carrying prophage λ*c*I857*S7* within the *bglB* gene located near the *unc* gene cluster [Miki, T., Hiraga, S., Nagata, T. & Yura, T. (1978) *Proc. Natl. Acad. Sci. USA* 75, 5099–5103]. When lysogenic cells carrying λ*asn* and λ*c*I857*S7* were induced at high temperature, synthesis of the F_1-ATPase portion of the complex increased to severalfold that of the noninduced cells. In contrast, no increase was observed upon thermoinduction of cells carrying λ*uncA* or λ*bglC*. The number of membrane sites that could bind purified F_1-ATPase also increased significantly upon induction by λ*asn* but not by λ*uncA* or λ*bglC*. In addition, F_1-depleted membranes prepared from λ*asn*-induced bacteria required more dicyclohexylcarbodiimide to seal the proton pathway than did those from noninduced bacteria. These results strongly suggest that λ*asn* carries a set of bacterial genes coding for all the F_1 polypeptides (the α, β, γ, δ, and probably the ε subunits) and at least some of the genes involved in formation of F_0 polypeptides. Although λ*uncA* carries the structural gene (*uncA*) for the α subunit of F_1-ATPase, it apparently does not carry the whole set of F_1–F_0 genes.**

The coupling factors of oxidative phosphorylation (F_1–F_0) that are found in various organelles, such as bacterial membranes and mammalian mitochondria, have similar structures (for review, see refs. 1–3). The catalytic portion of the complex, F_1-ATPase, which consists of five subunits (α, β, γ, δ, and ε) and is extrinsic to the membrane, has been purified from various sources and studied extensively. The other part (F_0) appears to be integrated into the membrane, forming a proton pathway in energy transduction. There are a few reports on purification of the entire complex (F_1–F_0) or the F_0 part (4–8). Recently, Sone *et al.* (8) found only two kinds of polypeptides in F_0 from a thermophilic bacterium and showed that these two subunits, binding sites for F_1 and dicyclohexylcarbodiimide (DCCD), mediated all the activities of F_0 when incorporated into liposomes. DCCD-binding proteins, as F_0 components, have been purified to homogeneity from other sources (9, 10).

Use of *Escherichia coli* as a source of the F_1–F_0 complex has the advantage in that this organism is well defined genetically. Mutations affecting this complex have been identified, and the structural genes for F_1 (*uncA,D*) (11, 12) and F_0 (*uncB,C* and *dcc*) (13–15) have been mapped around 83 min on the current linkage map (16). It has recently been shown by reconstitution assay (17) that *uncA* is the structural gene for the α subunit of F_1 (18, 19) and by membrane protein analysis that *uncD* is the structural gene for β subunit (20). Fillingame (15) has shown that *dcc* is the structural gene for the DCCD-binding protein in F_0. However, the structural genes for the other subunits have not been identified.

Specialized transducing phage carrying genes for the entire complex should be useful in determining the organization of these genes, and cells lysogenic for such phages should be useful in purification and characterization of the complex, provided that they can synthesize a larger amount of the complex upon induction. In this study, we used a set of specialized transducing phages recently isolated by Miki *et al.* (21) that carry segments of the bacterial chromosome around *uncA*. We found that increased synthesis of the coupling factor complex indeed occurred upon induction of cells carrying prophage λ*asn*, suggesting that this phage contains most of the genes coding for the polypeptides of the complex. In contrast, cells carrying prophage λ*uncA* did not show increased synthesis of the complex, suggesting that the latter phage carries only some of the genes for the complex.

EXPERIMENTAL PROCEDURES

Organism and Growth Conditions. The following λ lysogens were used: KY7405 (*bglC, lac, glmS*/λ*bglC c*I857S7/λ*c*I857S7), KY7241 (*uncA401, gal-1, lacY, tonA*/λ*uncA c*I857S7/λ*c*I857S7), and KY7485 (*asn31, thi, rif*/λ*asn c*I857S7/λ*c*I857S7). As described (21), these transducing phages carry different but overlapping segments of the *E. coli* chromosome: λ*bglC* (*bglC, R, glmS*), λ*uncA* (*bglC, R, glmS, uncA*), and λ*asn* (*bglC, R, glmS, uncA, asn*). Detailed genetic maps of these phages are shown in Fig. 1. KH716 (*asn31, thi, rif*) is the nonlysogenic parent strain of KY7485. *UncA401* in AN120 (11) and *uncB402* in AN382 (13) were transduced separately into KY2322 (*lac-1, gal-1, gal-2, ilv*^{ts}) and the transductants, KY2377 (*uncA401*) and OK101 (*uncB*), were used in this study.

Cells were grown aerobically at 30°C in semidefined medium (5.0 g of beef extract, 15.0 g of peptone, 5.0 g of NaCl, and 5.0 g of K_2HPO_4 per liter, pH 7.0) unless otherwise specified.

Induction of λ Phages. Logarithmic-phase cultures grown in the semidefined medium at 30°C were shifted to 42°C and shaken for 20 or 35 min, depending on the scale of the culture, to induce phage growth by inactivating the temperature-sensitive λcI repressor. Cultures were then shifted back to 37°C and incubation was continued for several hours with constant shaking.

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Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; DCCD, dicyclohexylcarbodiimide; F_1–F_0, coupling factor of oxidative phosphorylation.

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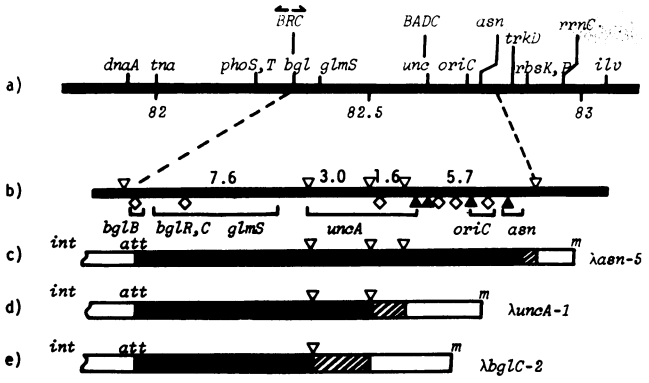

FIG. 1. *E. coli* chromosome segments carried by various λ phages. The *E. coli* linkage map around the *unc* region (*a*), the cleavage map of *E. coli* DNA by restriction endonucleases (*b*), the cleavage map of λ*asn* DNA (*c*), λ*uncA* DNA (*d*), and λ*bglC* DNA (*e*) are shown schematically. The positions of genes and genetic symbols on the genetic map are as described by Bachmann *et al.* (16) except that the positions of *asn*, *oriC*, and *tna* are as reported by Miki *et al.* (21). The gene positions on the physical map (21, 22) are taken from ref. 21. Open bar, λ DNA; solid bar, *E. coli* DNA (transducing segment); hatched bar, uncertain region of λ and *E. coli* DNA. Numbers show the sizes of each *Eco*RI segment in megadaltons. The cleavage sites of *Eco*RI (▽), *Bam*HI (▲), and *Hin*dIII (◇) are shown. For further details, see ref. 21.

Preparation of Membrane Vesicles and EDTA Extract. Cells were grown aerobically in 1 liter of medium to logarithmic phase (about 10^8 cells per ml) at 30°C, shifted to 42°C for 35 min, and then shaken at 37°C for 2 hr. Cells grown at 30°C without temperature shift were used as controls. Cells were collected by centrifugation, washed once, and resuspended in 20 ml of buffer A [10 mM Tris·HCl, pH 8.0/0.14 M KCl/2.0 mM 2-mercaptoethanol/10% (wt/vol) glycerol]. The cell suspension was passed through a French pressure cell (400 kg/cm², about 5700 psi or 39.3 MPa) and then undepleted membrane vesicles were prepared by the published procedure (23). To obtain membrane vesicles depleted of F_1-ATPase, membranes were suspended in 1.0 mM Tris·HCl, pH 8.0/0.5 mM EDTA/10% glycerol and centrifuged at 105,000 × *g* for 120 min. The resulting pellet (depleted membrane) was resuspended in buffer A (about 20 mg of protein per ml), and an equal volume of glycerol was added. The supernatant fraction (EDTA extract) contained about 85% of the F_1-ATPase originally present in the membranes.

Assay of Binding of ATPase to Depleted Membranes. Depleted membranes (0.25 mg of protein) and various amounts of purified F_1-ATPase were mixed in 300 μl of buffer A containing 5.0 mM $MgCl_2$ and incubated at 22°C for 15 min. Then the membrane vesicles with re-bound F_1 were collected by centrifugation at 105,000 × *g* for 60 min and washed once with buffer A. The pellet was suspended in buffer A containing 2.0 mM $MgCl_2$ and an equal volume of glycerol was added. ATPase was assayed as described below.

Measurement of Formation of a Proton-Motive Force. Formation of a proton-motive force in the membrane vesicles was estimated by the quenching of quinacrine fluorescence as described (24). Membrane vesicles (100–200 μg of protein) suspended in 2.0 ml of 20 mM tris(hydroxymethyl)methylamine, pH 8.0 (KOH/100 mM KCl/2.0 mM $MgCl_2$ were mixed with 10 μl of 50 μM quinacrine, and fluorescence (emission, 500 nm; excitation, 420 nm) was monitored in a Hitachi spectrofluorometer, model MPF4. Then, 10 μl of 1.0 M potassium succinate was added, and respiration-dependent quenching of fluorescence was recorded.

Polyacrylamide Gel Electrophoresis. EDTA extracts of the membranes obtained as described above were analyzed by electrophoresis in either 12.5% polyacrylamide gel (25), the same gel containing 8.0 M urea (10), or a gradient gel (12–25% polyacrylamide gel) (26). All systems contained 0.1% sodium dodecyl sulfate ($NaDodSO_4$) and were run in a slab gel apparatus. Samples were incubated in 1.0% $NaDodSO_4$/2.0% 2-mercaptoethanol for 5 min in a boiling water bath before electrophoresis.

Assay Procedures. ATPase activity was assayed by measuring release of inorganic phosphate from ATP; 1 unit of enzyme was defined as the amount hydrolyzing 1 μmol of ATP per min under standard conditions (26). Protein was measured by the method of Lowry *et al.* (27).

Materials. F_1-ATPase was purified from *E. coli* ML308-225 as described (28). Chemicals were obtained as follows: ATP, Boehringer Mannheim; quinacrine, Sigma; reagents for polyacrylamide gel electrophoresis, Wako Chemical Co. (Tokyo, Japan). Other reagents were as described (17, 19, 28), or were the highest grade commercially available.

RESULTS

Genetics. The presence of *uncA* and *uncB* genes on λ*asn* and λ*uncA* (but not on λ*bglC*) was shown by the abilities of these phages to convert cells of both KY2377 (*uncA401*) and OK101 (*uncB402*) to Unc^+ by lysogenization. Recent studies from two laboratories have indicated that *uncA401* affects the structural gene for the α subunit of F_1 (18, 19). Thus, λ*asn* and λ*uncA* carry the structural gene for the α subunit. The existence of structural genes for other polypeptides of the F_1–F_0 complex on these phages has not been examined. The segments of *E. coli* chromosomes carried by these phages are shown in Fig. 1 and described above.

Increased F_1-ATPase Activity upon Thermoinduction of λ*asn* Prophage. The F_1-ATPase activities of cells lysogenic for each of the transducing phages were measured before and after induction of prophage at high temperature. As shown in Fig. 2, the specific activity of the enzyme in membranes from noninduced cells remained constant throughout the experiment and was twice as high in strain KY7485 (λ*asn*) as in strain KY7241 (λ*uncA*) or KY7405 (λ*bglC*). The specific activity of the enzyme in KY7485 (λ*asn*) was also about 3 times as high as in KH716 (parent strain of KY7485). These results suggest that KY7485 (λ*asn*) has at least two sets of genes for F_1-ATPase. In contrast, after thermoinduction of the λ*asn* prophage, the specific activity of the membrane enzyme increased linearly with time of incubation, reaching about 4-fold the level before induction. A treatment period of 20 min at 42°C was enough to obtain the maximal increase of specific activity. Because this increase was due to an increase in F_1 proteins, as shown below, the content of F_1 proteins in membranes from fully induced cells is calculated from this result to be about 0.04 mg/mg of membrane protein, assuming that the specific activity of the pure enzyme is 100 units/mg of protein (28). The true F_1 content of cytoplasmic membranes is probably much higher than this, because the membrane fractions used in this study consisted of mixtures of outer and cytoplasmic membranes. No significant increase in specific activity of the enzyme in the cytoplasmic fraction was observed during the experiment, suggesting that thermoinduction caused increased synthesis of the enzyme and then its assembly into the membranes. In sharp contrast, the F_1-ATPase activity associated with membranes did not increase on induction of prophage λ*uncA* or λ*bglC*.

Increased Synthesis of F_1-ATPase Proteins on Thermal Induction of Prophage λ*asn*. The same set of lysogen was grown on a large scale (1 liter) and membranes were prepared

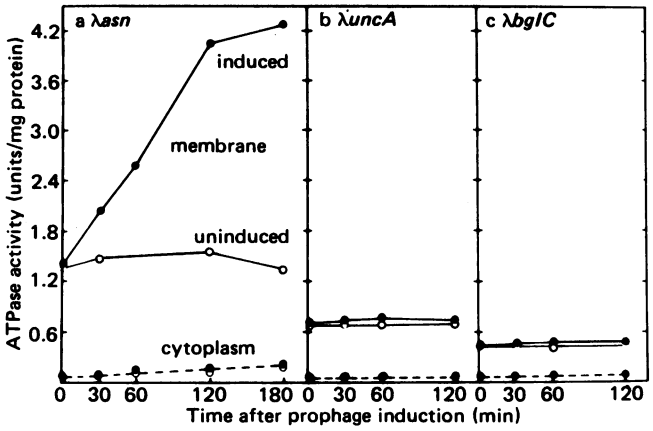

FIG. 2. Effect of thermoinduction of λ prophage on membrane ATPase activity in three lysogens. Each strain was grown in 80 ml of medium at 30°C with vigorous shaking. In the late logarithmic phase (about 6×10^8 cells per ml), the culture was divided into two portions. One portion (40 ml) was shifted to 42°C (●) for 20 min and then back to 37°C for further incubation. The other 40-ml portion (○), serving as a noninduced control, was shaken at 30°C without a temperature shift. Samples (4.0 ml) were taken from each culture at the times indicated and the cells were harvested, washed with 50 mM of Tris·HCl, pH 8.0/10 mM $MgCl_2$/50 μg of chloramphenicol per ml, and finally suspended in 3.0 ml of 50 mM Tris·HCl, pH 8.0/10 mM $MgCl_2$/10% glycerol/0.1 mM ATP. The suspension was sonicated intermittently at 20 kHz for a total of 3 min in an ice bath and then centrifuged at 105,000 × *g* for 60 min after removal of undisrupted cells. The specific activities of F_1-ATPase in the supernatant (cytoplasm; dotted lines) and pellet (membrane; solid lines) were determined. Numbers of helper phages (λcI857*S7*) per cell after thermoinduction were 19 in KY7241, 4 in KY7485, and 4 in KY7405. Number of λ*asn* was about 16 per cell after thermoinduction, judging from the distribution of phage particles in CsCl gradient centrifugation. Numbers of λ*uncA* and λ*bglC* could not be determined because they could not be separated from helper phages.

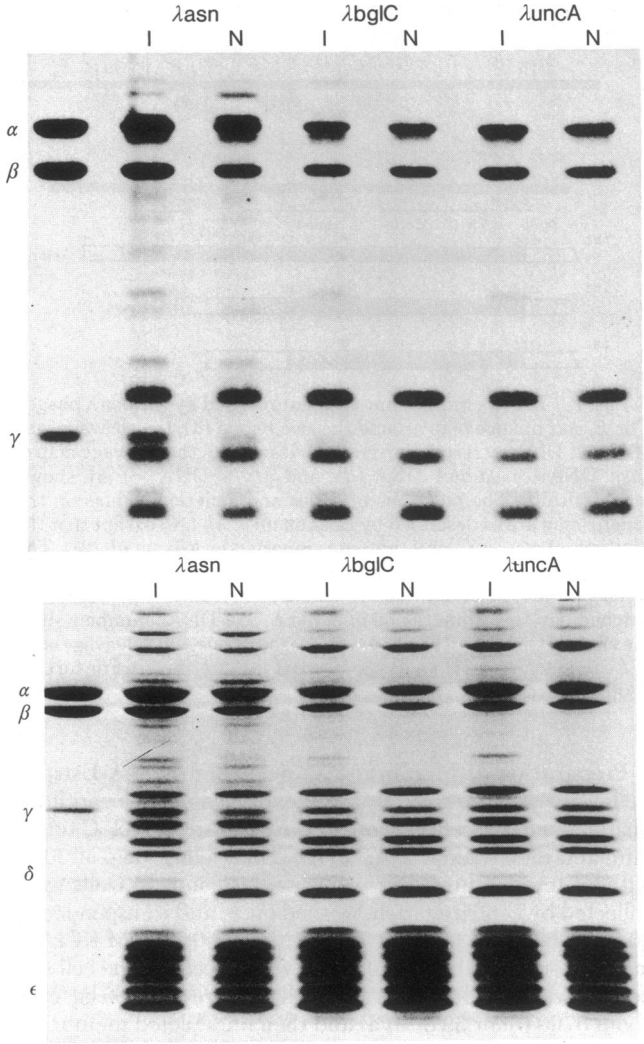

FIG. 3. Polyacrylamide gel electrophoresis of crude F_1-ATPase fractions (EDTA extract) from induced and noninduced cells. Cells were grown in 1 liter of medium and membranes were obtained from induced (I) and noninduced (N) cells. The following ATPase activities (in units/mg of protein) were found in the membranes: KY7405 induced, 1.1; KY7405 noninduced, 0.93; KY7421 induced, 1.1; KY7421 noninduced, 1.4; KY7485 induced, 4.1; KY7485 noninduced, 1.4. Crude ATPase fractions (EDTA extracts) were obtained from these membranes, treated with 2.0% 2-mercaptoethanol and 1.0% $NaDodSO_4$ in a boiling water bath, and analyzed by electrophoresis either in 12.5% gel (*Upper*) or in a gradient gel (*Lower*). Protein bands were stained with Coomassie brilliant blue. The amounts of protein applied were 26 μg (*Upper*) and 68 μg (*Lower*). As a control, purified F_1-ATPase was run simultaneously and the positions of its subunits are indicated (at the left).

from noninduced and fully induced cells. When the membranes were washed with EDTA, about 85% of their F_1-ATPase activity was solubilized. Samples of the EDTA extract were treated with $NaDodSO_4$ and subjected to polyacrylamide gel electrophoresis in the presence of this detergent. As shown in Fig. 3, thermoinduction of λ*asn* prophage caused significant increase in the amounts of membrane-associated F_1 polypeptides (α, β, γ, and δ) over the amounts in noninduced cells. This increased synthesis was apparently specific for F_1 subunits because no appreciable differences were found in staining densities of other protein bands from induced and control membranes. These results were confirmed by polyacrylamide gel electrophoresis in the presence of 8.0 M urea and 0.1% $NaDodSO_4$ (10). Although no increased synthesis of the ε subunit could be detected with the present gel electrophoresis (Fig. 3 *lower*), this subunit was probably synthesized stoichiometrically with the other subunits for the following reasons. First, most of the F_1 proteins were located in the membrane fraction both before and after thermoinduction, and both δ and ε are required for binding of the αβγ complex to membranes (29). Second, F_1-ATPase purified from induced λ*asn* lysogen by the published procedure (28) had essentially the same specific activity and subunit composition as F_1-ATPase obtained from ML308-225 (five-subunit enzyme) (data not shown). It is noteworthy that, when equal amounts of the two preparations were applied to gel (12–25% gradient of polyacrylamide), the bands of the ε subunits were of equal intensity.

These results suggest that λ*asn* carries an entire set of structural genes for F_1-ATPase and that strain KY7485 can synthesize all the F_1 subunits more rapidly after thermoinduction of the prophage λ*asn*. As shown in Fig. 3, other strains (KY7241 and KY7405) did not show any increase in synthesis of F_1 subunits (in EDTA-extracts of the membranes) after induction of the respective prophages.

Increased Capacity of Membranes to Bind F_1-ATPase after Prophage Induction. Because most of the F_1 molecules synthesized after thermoinduction of λ*asn* were found in the membrane fraction, at least some membrane components (F_0), such as the binding sites for F_1, are probably synthesized in increased amounts after prophage induction. This expectation was confirmed by studying the binding of purified F_1-ATPase to "depleted membranes" obtained from induced and noninduced cells. Membranes were first washed with EDTA to remove F_1 and then incubated with purified F_1. As shown in Fig. 4*a*, depleted membranes from induced cells could bind about

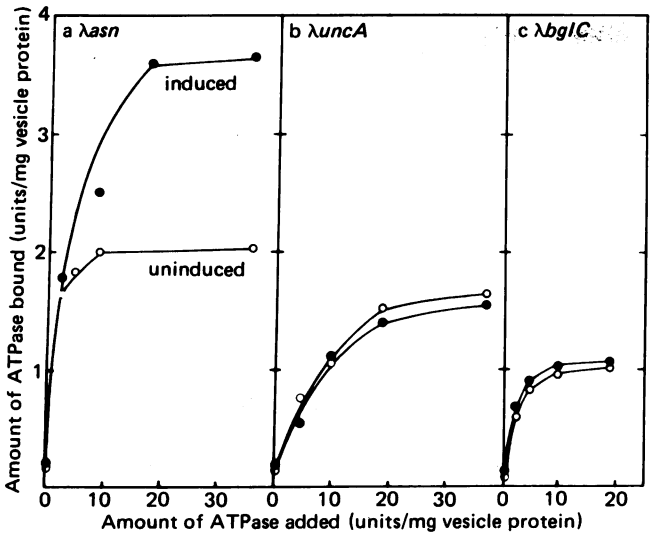

FIG. 4. Binding of purified F_1-ATPase to depleted membrane vesicles from induced and noninduced cells. Cells were grown in 1 liter of medium and membranes were obtained from induced (●) and noninduced (○) cells. The membranes were washed with buffer containing EDTA to deplete them of F_1-ATPase and then incubated with different amounts of purified F_1-ATPase. The ATPase activity rebound to the membranes was assayed.

twice as much F_1 as the control membranes. F_1-ATPase molecules re-bound to the membrane were active in forming an ATP-driven proton-motive force in the membrane vesicles, as judged by measuring quenching of quinacrine fluorescence (data not shown). No such increase of F_1 binding sites was observed after prophage induction in the two other strains tested (Fig. 4 *b* and *c*).

Increase of Proton Pathway after λ*asn* Prophage Induction. The F_0 part of the coupling factor is known to serve as a proton pathway (2–4). Membranes depleted of F_1-ATPase are known to show leakage of protons, and this leakage can be prevented by binding of F_1 or DCCD to the F_0 portion (2–4, 30, 31). Thus, the amount of the F_0 part (at least the proton ionophore part) can be estimated by measuring the amount of DCCD required to stop proton leakage and to form a proton-motive force in depleted membrane vesicles.

In this study, quenching of fluorescence of quinacrine was measured as an index of the proton-motive force formed by respiration (24). Respiratory-driven quenching could be restored by the binding of F_1 or DCCD to depleted membranes. Thus, the degree of restoration of the respiratory-driven reaction by DCCD was followed as a measure of the proton pathways. Succinate-driven quenching was not observed with 1 μM DCCD in depleted membranes from thermoinduced cells of KY7485 (λ*asn*), whereas substantial quenching was observed in those from noninduced cells (Fig. 5*a*). The extent of stimulation of quenching was always higher in control vesicles than in induced vesicles, when compared at low concentrations of DCCD that did not cause nonspecific inhibition of quenching. At higher DCCD concentrations (>20 μM), the apparent quenching by both types of vesicles reached essentially the same value, but comparisons should be made at DCCD concentrations of less than 10 μM because concentrations >10 μM inhibited the quenching of undepleted membrane vesicles (Fig. 4*b*). It is evident that, for equal quenching under these conditions, membranes from λ*asn*-induced cells required a higher concentration of DCCD than did those from control cells. This is consistent with the notion that the induced cells synthesized increased amounts of F_0 polypeptides.

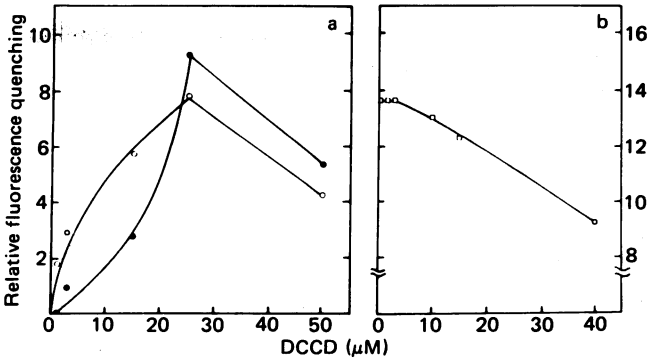

FIG. 5. Effect of concentration of DCCD on succinate-driven quenching of quinacrine fluorescence. (*a*) Membranes were prepared from induced (●) and noninduced (○) cells and washed with buffer containing EDTA to deplete them of F_1-ATPase. Quenching of quinacrine fluorescence (succinate-dependent) was measured after addition of DCCD at various concentrations to 140 μg of membrane vesicle protein. The extent of fluorescence change reached a plateau in 4.5 min, and this plateau value is plotted against the concentration of DCCD. Other conditions were as described in the text. (*b*) Unwashed membrane vesicles (140 μg of protein per assay) were prepared from noninduced cells (grown at 30°C). After quenching of fluorescence (succinate-dependent) had reached a maximal value, various concentrations of DCCD were added. The quenching decreased and reached another plateau value. The value reached at 4.5 min after addition of DCCD is plotted against the concentration of DCCD. The extent of fluorescence quenching was expressed by the same relative scale as in *a*. Other conditions were as for *a* or as described in the text.

DISCUSSION

The results presented above indicate that *E. coli* strain KY7485 (λ*asn*) synthesized increased amounts of F_1 polypeptides (α, β, γ, δ, and probably ε) and at least part of F_0 (binding sites for F_1 and a DCCD-sensitive component of the proton pathway) upon thermoinduction of the prophage. Recently, Sone *et al.* (8) have shown that only two components (the binding sites for F_1 and DCCD) from a thermophilic bacterium are sufficient to reconstitute active F_0 functions in liposomes. Thus, it appears probable that all the F_0 components were synthesized in increased amounts after prophage induction. These results suggest that λ*asn* carries an entire set of structural genes for F_1 and F_0 polypeptides. On the other hand, no increased synthesis of any of the components of the F_1–F_0 complex could be detected in membrane fractions of KY7241 (λ*uncA*) or KY7405 (λ*bglC*) after prophage induction. However, the genes for the α subunit located on λ*uncA* must be active in strain KY7241 before thermoinduction, because this strain can synthesize active F_1-ATPase. Possibly, synthesis of α subunits did actually increase after thermoinduction of prophage λ*uncA*, but no increase could be detected because these subunits were in the cytoplasm or were rapidly degraded. No evidence has yet been obtained that λ*bglC* has any of the structural genes for the F_1–F_0 complex.

Recent genetic work by Gibson *et al.* (32) showed that four *unc* genes represent part of the single operon and are transcribed as a unit in the order *uncB*, *uncA*, *uncD*, and *uncC* on the *E. coli* chromosome. In this study we have shown that λ*asn* and λ*uncA* have *uncB* and *uncA* genes. Phage λ*asn* also seems to carry *uncD* and *uncC* genes because it carries genes for all the F_1 and F_0 components as discussed above.

The restriction endonuclease cleavage map of the transducing phages used here (21) (Fig. 1) suggests that most of the structural genes for the F_1–F_0 complex are located in three DNA fragments obtained by digestion with *Eco*RI endonuclease: 1.7- and 3.1-megadalton fragments containing *uncA*,

and a 5.7-megadalton fragment containing *asn* carried by λ*asn* but not by λ*uncA*. Systematic analysis of mutations in these regions of the chromosome should give more information on the organization and expression of the *unc* gene cluster(s).

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1. Senior, A. E. (1973) *Biochim. Biophys. Acta* **301**, 249–277.
2. Harold, F. M. (1976) *Curr. Top. Bioenerg.* **6**, 83–149.
3. Racker, E. (1976) *A New Look at Mechanisms in Bioenergetics* (Academic, New York).
4. Okamoto, H., Sone, N., Hirata, H., Yoshida, M. & Kagawa, Y. (1977) *J. Biol. Chem.* **252**, 6125–6131.
5. Serrano, R., Kanner, B. I. & Racker, E. (1976) *J. Biol. Chem.* **251**, 2453–2461.
6. Tzagoroff, A. & Meagher, P. (1971) *J. Biol. Chem.* **246**, 7328–7336.
7. Steckhoven, F. S., Waitkus, R. F. & van Moerkerk, H. T. B. (1972) *Biochemistry* **11**, 1144–1150.
8. Sone, N., Yoshida, M., Hirata, H. & Kagawa, Y. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4219–4223.
9. Cattel, K. J., Knight, I. G., Lindop, C. R. & Beechey, R. B. (1970) *Biochem. J.* **125**, 169–177.
10. Fillingame, R. H. (1976) *J. Biol. Chem.* **251**, 6630–6637.
11. Butlin, J. D., Cox, G. B. & Gibson, F. (1971) *Biochem. J.* **124**, 75–81.
12. Cox, G. B., Downie, J. A., Gibson, F. & Radik, J. (1978) *Biochem. J.* **170**, 593–598.
13. Butlin, J. D., Cox, G. B. & Gibson, F. (1973) *Biochim. Biophys. Acta* **292**, 366–375.
14. Gibson, F., Cox, G. B., Downie, J. A. & Radik, J. (1977) *Biochem. J.* **164**, 193–198.
15. Fillingame, R. H. (1975) *J. Bacteriol.* **124**, 870–883.
16. Bachmann, B., Low, K. B. & Taylor, A. L. (1976) *Bacteriol. Rev.* **40**, 116–167.
17. Futai, M. (1977) *Biochem. Biophys. Res. Commun.* **79**, 1231–1237.
18. Dunn, S. D. (1978) *Biochem. Biophys. Res. Commun.* **82**, 596–602.
19. Kanazawa, H., Saito, S. & Futai, M. (1978) *J. Biochem.* **84**, 1513–1517.
20. Fayle, D. R. H., Downie, J. A., Cox, G. B., Gibson, F. & Radik, J. (1978) *Biochem. J.* **172**, 523–531.
21. Miki, T., Hiraga, S., Nagata, T. & Yura, T. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5099–5103.
22. Marsh, R. C. & Worcel, A. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2720–2724.
23. Tsuchiya, T. & Rosen, B. P. (1975) *J. Biol. Chem.* **250**, 7687–7692.
24. Tsuchiya, T. & Rosen, B. P. (1975) *J. Biol. Chem.* **250**, 8409–8415.
25. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
26. Studier, F. W. (1973) *J. Mol. Biol.* **79**, 237–248.
27. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
28. Futai, M., Sternweis, P. C. & Heppel, L. A. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 2725–2729.
29. Sternweis, P. C. (1978) *J. Biol. Chem.* **253**, 3123–3128.
30. Rosen, B. P. (1973) *Biochem. Biophys. Res. Commun.* **53**, 1289–1296.
31. Altendorf, K., Harold, F. M. & Simoni, R. D. (1974) *J. Biol. Chem.* **249**, 4587–4593.
32. Gibson, F., Downie, J. A., Cox, G. B. & Radik, J. (1978) *J. Bacteriol.* **134**, 728–736.