Uncoupling protein mediates electrophoretic transport of protons and anions across the inner membrane of brown adipose tissue mitochondria. The mechanism and site of proton transport, the mechanism by which fatty acids activate proton transport, and the relationship between fatty acids and anion transport are unknown. We used fluorescent probes to measure $H^+$ and anion transport in vesicles reconstituted with purified uncoupling protein and carried out a comparative study of the effects of laurate and its close analogue, undecanesulfonate. Undecanesulfonate was transported by uncoupling protein with a $K_m$ value similar to that observed for laurate as it activated $H^+$ transport. Both laurate and undecanesulfonate inhibited $Cl^-$ with competitive kinetics. Undecanesulfonate inhibited laurate-induced $H^+$ transport with competitive kinetics. Undecanesulfonate and laurate differed in two important respects. (i) Laurate caused uncoupling protein-mediated $H^+$ transport, whereas undecanesulfonate did not. (ii) Lauric acid was rapidly transported across the bilayer by nonionic diffusion, whereas undecanesulfonic acid was not. We infer that the role of uncoupling protein in $H^+$ transport is to transport fatty acid anions and that fatty acids induce $H^+$ transport because they can diffuse electroneutrally across the membrane. According to this hypothesis, uncoupling protein is a pure anion porter and does not transport protons; rather it is designed to enable fatty acids to behave as cycling protonophores.

The mitochondrial uncoupling protein (UCP) is a remarkable chemiosmotic device engineered to dissipate the proton-motive energy of brown adipose tissue mitochondria and provide heat to the animal. It is known that UCP-mediated uncoupling is activated by fatty acids, and the consensus has been that UCP is a $H^+$ (or $OH^-$) transporter (1–3). It is also known that UCP, like other members of its gene family, is an anion transporter (1, 2, 4–6). Both $H^+$ and anion transport are inhibited by purine nucleotides.

The mechanism by which FAs activate proton transport via UCP is unknown. We have long considered UCP-mediated anion transport to hold the key to this mystery, not least because this property has been difficult to integrate with the physiological function of UCP. We have recently shown that FAs inhibit $Cl^-$ transport through UCP with competitive kinetics (7), supporting our hypothesis that anions are transported through the FA binding domain of UCP (8). The close relationship between FA interaction and anion transport permits at least two possibilities for the mechanism of $H^+$ transport. Either FAs activate proton transport while being anchored within the UCP (9) or FA anions are directly transported by UCP (10, 11). FA anion transport by UCP would not be unexpected, in view of the fact that UCP transports monovalent anions generally and transport rates increase with anion hydrophobicity (5, 6).

To distinguish between these possibilities, we compared the properties of laurate and its close analogue, undecanesulfonate. We measured fluxes of $H^+$, $Cl^-$, and $K^+$ in proteoliposomes reconstituted with purified UCP (5). $K^+$ flux, in the presence of valinomycin, was used as a measure of anionic or protonic charge flux. Both undecanesulfonate and laurate are competitive inhibitors of UCP-mediated $Cl^-$ transport, and undecanesulfonate is a competitive inhibitor of laurate-induced $H^+$ transport. Both anions catalyzed charge movement across the proteoliposomal membrane, and they did so with hyperbolic kinetics and with similar $K_m$ and $V_{max}$ values. In the case of laurate, the charge movement was due to $H^+$ flux. Undecanesulfonate did not catalyze $H^+$ flux, from which it follows that it is transported as the anion by UCP, as are other alkylsulfonates (6). This difference in behavior of the two analogues in proteoliposomes could be fully explained by their behavior in protein-free liposomes; laurate supported rapid electroneutral $H^+$ transport across the bilayer due to nonionic flip-flop of the FA. Undecanesulfonate, a strong acid, was unable to catalyze electroneutral $H^+$ transport. We conclude that FAs cause electrophoretic $H^+$ transport in the presence of UCP because UCP is an anion channel designed for electrophoretic transport of FA anions. According to this mechanism, UCP-mediated uncoupling is due to futile cycling of FAs across the inner membrane of brown adipose tissue mitochondria. Seen in this light, UCP-transported monovalent anions are accidental substrates of the FA anion pathway in UCP.

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1 The abbreviations used are: UCP, uncoupling protein; octyl-POE, n-octylpolyoxyethylene; FA, fatty acid; PBFI, potassium-binding benzofuran isophthalate; SPQ, 6-methoxy-N-(3-sulfopropyl)quinolinium; TEA, tetraethylammonium cation; TES, N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid.

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2 A preliminary report of these results has been published as an abstract (11).
EXPERIMENTAL PROCEDURES

Purification and Reconstitution of UCP—Brown adipose tissue mitochondria were isolated from Syrian hamsters, and UCP was purified and reconstituted into proteoliposomes using protocols described previously (12). Where used, protein-free liposomes were prepared by the same protocol. When fresh mitochondria were used, H⁺ flux in proteoliposomes was nearly identical to the background rates observed in liposomes, indicating that Bio-Beads SM-2 removed virtually all endogenous FAs (9). When frozen mitochondria were used, a higher, GDP-inhibited H⁺ flux was observed in the absence of added FAs. Accordingly, frozen mitochondria were first washed with 5 mg of bovine serum albumin/ml, which eliminated this effect.

Quantification of Ion Fluxes in Liposomes and Proteoliposomes—Media compositions were designed to set up an electrochemical K⁺ gradient, favoring transport of anions or H⁺ in the appropriate direction when valinomycin was added. Fluorescence was assayed in cuvettes containing 0.5 mg of lipid/ml and UCP at 1-3 μg of protein/mg of lipid. Intraliposomal volume, estimated from the volume of distribution of probe, was 1.1-1.6 μl/mg of lipid.

The data presented in this paper are based on flux measurements of three ions. Measurement of UCP-mediated H⁺ flux was obtained from changes in SPO fluorescence due to quenching by the anion of TES buffer (12, 13). Internal medium contained 84.4 mM TEA·SO₄, 0.6 mM TEA-EGTA, and 28.8 mM TEA-TES, pH 7.2. External medium contained 60 mM K₂SO₄, 24.4 mM TEA·SO₄, 0.6 mM TEA-EGTA, and 28.8 mM TEA-TES, pH 7.2. Measurement of UCP-dependent K⁺ flux, reflecting the movement of ionic charge across the membrane (14), was obtained from changes in PBFI fluorescence (12, 15). Except for different probes, internal, and external media were identical to those used for H⁺ flux. Measurement of UCP-mediated Cl⁻ flux was determined from changes in SPO fluorescence due to Cl⁻ quenching (5, 12). Internal medium contained 79.5 mM TEA·SO₄, 0.6 mM TEA-EGTA, and 20 mM TRIS-PO₄, pH 7.2. External medium contained 119.25 mM KCl, 0.6 mM TEA-EGTA, and 20 mM TRIS-PO₄, pH 7.2.

Evaluation of Lytic Effects of Laurate and Undecanesulfonate in Liposomes and Proteoliposomes—We investigated nonspecific effects of FAs and long chain sulfonates and obtained the following results (data not shown). (i) Laurate and undecanesulfonate were poor lytic agents, in that neither induced liposomal lysis, even at levels up to 1 mM. Lysis, detected when internal probe was exposed to external medium, was apparent with high doses of longer chain FAs or alkylsulfonates. (ii) The Kᵢ for GDP-inhibition of H⁺ or Cl⁻ transport in proteoliposomes was unaffected by the amount of FA present during the assay (7). (iii) Laurate caused increases in the rates of valinomycin-induced H⁺ or Cl⁻ transport in liposomes. This effect was amplified by increasing valinomycin concentration, suggesting ion pair transport of laurate with the valinomycin-K⁺ complex (16). At 100 mM, the amount of valinomycin used in these experiments, this effect was small, linear with [laurate], and scarcely detectable below 50 μM laurate. In general, this effect was observed with all amphiphiles. In our experience the nonspecific transport could be distinguished by its insensitivity to GDP.

Materials—SPO and PBFI were purchased from Calbiochem and Molecular Probes, respectively. Essentially FA-free bovine serum albumin (Factor IV), FAs, and ionophores were purchased from Sigma. Alkylsulfonates were purchased from Research Plus, Inc. Materials for protein purification and liposome formation were obtained from sources described in Juzek et al. (5).

RESULTS

Undecanesulfonate and Laurate Induce GDP-sensitive, Electrophoretic Fluxes in Liposomes Reconstituted with UCP—The traces in Fig. 1A follow valinomycin-induced H⁺-efflux from proteoliposomes containing UCP and treated with 10 μM laurate. H⁺ transport was measured directly from quenching of SPQ fluorescence by TES anion. The traces in Fig. 1B were obtained under identical conditions except that H⁺ transport was measured indirectly as K⁺ uptake, determined from PBFI fluorescence. The traces in Fig. 1C also follow K⁺ uptake determined from PBFI fluorescence; however, undecanesulfonate was used instead of laurate to induce UCP-dependent ion movement.

An acidification jump can be seen following addition of laurate when H⁺ flux was measured (20–24 s, Fig. 1A). This reflects rapid equilibration of lauric acid across the membrane. A capacitative K⁺ jump can be seen following addition of valinomycin when K⁺ flux was measured (Fig. 1B and C).

These experiments establish several key aspects of UCP behavior. (i) The observed fluxes were electrophoretic, because they required valinomycin and a K⁺ gradient. (ii) The observed fluxes required addition of 10–100 μM of a long chain FA or alkylsulfonate. In their absence, observed fluxes were nearly...
rates. These differential effects on H^+ efflux are determined from changes in intraliposomal [TES^+]_i, plotted versus concentration ([Anion]) of undecanesulfonate (●) and laurate (○). Laurate or undecanesulfonate were added to the proteoliposomes in assay medium, followed by addition of 0.1 μM valinomycin to initiate H^+ efflux, as in Fig. 1A. Net fluxes are plotted after subtraction of flux obtained in the presence of 1 mM external GDP. Nonlinear regression (solid line) of the dose-response curve yielded K_m, of 9 μM for laurate. For undecanesulfonate, the dashed line merely connects the points. Media compositions were identical for the two anions and are described under “Experimental Procedures.”

The Laurate Analogue, Undecanesulfonate, Is Transported by UCP but Does Not Catalyze UCP-Mediated H^+ Flux—We measured proton flux in proteoliposomes reconstituted with UCP as a function of [laurate] and [undecanesulfonate], and the results are plotted in Fig. 2. Undecanesulfonate (Fig. 2, triangles) was incapable of catalyzing UCP-dependent H^+ flux and even inhibited the small endogenous H^+ flux. Laurate, in contrast, induced profound activation of H^+ efflux (Fig. 2, circles) in proteoliposomes reconstituted with UCP, with an apparent K_m in the micromolar range.

As shown in Fig. 1, B and C, both laurate and undecanesulfonate catalyzed net charge movement via UCP at comparable rates. These differential effects on H^+ transport were confirmed in swelling experiments on intact brown adipose tissue mitochondria (not shown). The results of these experiments support two important conclusions. The behavior of undecanesulfonate differs from that of its close analogue, laurate, and measurements of K^+ flux under the conditions of Fig. 1C are reporting influx of the undecanesulfonate anion and not efflux of H^+.

Comparative Kinetics of Undecanesulfonate Influx and Laurate-Induced H^+ Efflux in Proteoliposomes Reconstituted with UCP—Fig. 3 contains Eadie-Hofstee plots of undecanesulfonate influx and of laurate-catalyzed H^+ efflux. Fluxes were identical with trace d in each figure (see below). (iii) The observed fluxes required UCP and were not observed in liposomes lacking UCP (not shown). (iv) Fluxes were completely inhibited when GDP was present on both sides of the membrane. Thus, traces from identical experiments in liposomes lacking UCP were superimposable on the lowest traces of each panel in Fig. 1. (v) The pattern of GDP-sensitivity of fluxes induced by 10^{-5} M laurate and 10^{-5} M undecanesulfonate (Fig. 1) is entirely similar to that previously observed for flux induced by 10^{-3} M Cl^- (2). Thus, these fluxes were partially inhibited when GDP was present on one side of the membrane and fully inhibited when GDP was present on both sides. This reflects the fact that the GDP binding site is accessible only from one side of the protein (17) and UCP is more or less randomly inserted into the liposomal membrane (5). (vi) K^+ flux in the presence of valinomycin is a reliable measure of charge movement through UCP.

Undecanesulfonate and Laurate Are Competitive Inhibitors of Cl^- Flux through UCP—We have shown previously that laurate is a competitive inhibitor of Cl^- transport through UCP (7). The data in Fig. 4 show that undecanesulfonate is also a potent inhibitor of Cl^- transport through UCP. The effects of these analogues on the kinetics of Cl^- uptake are compared in Fig. 5. Competitive inhibition by both anions is demonstrated by the parallel Hanes plots (Fig. 5). This result extends previous findings that short and medium chain alkylsulfonates are competitive with Cl^- transport (6).

Undecanesulfonate Is a Competitive Inhibitor of Laurate-Induced H^+ Efflux in Proteoliposomes Reconstituted with UCP—Anions that do not quench SPO fluorescence (13) can be investigated for their effects on H^+ transport because they do not interfere with the measurement. NO_3^- (18) and hexanesulfonate (6) are transported electropheretically by UCP, and we have shown that they are competitive inhibitors of laurate-induced H^+ transport (7). The double-reciprocal plots in Fig. 6 show that undecanesulfonate is also competitive with laurate as an inhibitor of UCP-mediated H^+ transport.

Undecanesulfonate Is Incapable of Nonionic Diffusion in Liposomes—The preceding results show that undecanesulfonate and laurate behave similarly with respect to UCP. The notable exception is that laurate causes UCP-mediated proton transport, whereas undecanesulfonate does not. The likely structural basis for this exception is that FAs are weak acids, whereas the alkyl sulfonic acids are very strong acids, with pK_a ~ 0 (19). Accordingly, lauric acid is more likely than undecanesulfonic acid to undergo nonionic diffusion across the membrane.
Transport Mechanism of Uncoupling Protein

The table contains $V_{max}$, $K_m$, and limiting permeabilities for a series of anions whose kinetics have been measured using UCP reconstituted into proteoliposomes. Limiting permeability is flux/[anion] as [anion] approaches zero and equals the ratio, $V_{max}/K_m$. SPQ fluorescence quenching was used to measure flux of Cl$^-$ and flux of H$^+$ induced by laurate and oleate. PBFI fluorescence was used to measure K$^+$ flux as an indirect measure of hexanesulfonate and undecanesulfonate flux. $\Delta \psi$ varied between 120 and 140 mV for these measurements, and parameters have not been adjusted for the effects of these variations.

The traces in Fig. 7 demonstrate that lauric acid equilibrates rapidly across the membrane, resulting in the delivery of protons to the intraliposomal space of protein-free liposomes. In contrast, undecanesulfonate additions were without effect on intraliposomal pH. Thus, undecanesulfonate acid is not transported by nonionic diffusion, probably because its concentration nearly vanishes near neutral pH.

**FIG. 4.** Laurate and undecanesulfonate inhibit Cl$^-$ influx into proteoliposomes reconstituted with UCP. Percent inhibition of UCP-mediated Cl$^-$ influx is plotted versus [laurate] (●) and [undecanesulfonate] ( ). Nonlinear regressions (solid lines) yielded $K_m$ values of 23 and 32 μM for undecanesulfonate and laurate, respectively. Hill coefficients for both curves are 1. Cl$^-$ flux was determined from SPQ fluorescence as described in Experimental Procedures.

**FIG. 5.** Laurate and undecanesulfonate are competitive inhibitors of UCP-mediated Cl$^-$ transport. UCP-mediated Cl$^-$ influx was measured as described under "Experimental Procedures." $J_{\text{Cl}^-}$ was measured in varying Cl$^-$ concentrations ([Cl$^-$]) without additions (●) and containing 10 μM laurate (●) or 50 μM undecanesulfonate (●). [Cl$^-$] was varied by mixing medium containing 175.5 mM KCl with medium containing 175.5 mM potassium gluconate. Internal medium was adjusted with TEA$\text{SO}_4$ to be isosmotic with external medium. Cl$^-$ uptake was initiated with 0.1 μM valinomycin. Linear regressions (solid lines) of the initial rates yielded apparent $K_m$ values for Cl$^-$ of 140 μM (●), 164 μM (●), and 332 μM (●). Corresponding $V_{max}$ values (nmol of Cl$^-$/min mg of protein) were 9490 (●), 8800 (●), and 8530 (●). Assuming fully competitive inhibition, the $K_m$ values calculated for undecanesulfonate and laurate were 37 and 66 μM, respectively.

**FIG. 6.** Undecanesulfonate is a competitive inhibitor of laurate-induced H$^+$ efflux in proteoliposomes reconstituted with UCP. The dependence of H$^+$ efflux on [laurate] is expressed as a double-reciprocal plot without further addition (●) and in the presence of 100 μM undecanesulfonate (●). $J_{\text{H}^+} - J_{\text{H}^+}$ is the difference in flux in the presence (●) and absence (○) of laurate. H$^+$ efflux was measured as described under "Experimental Procedures." Linear regressions of the data (solid lines) yielded apparent $K_i$ values for laurate of 22 and 53 μM in the absence and presence of 100 μM undecanesulfonate, respectively. Based on purely competitive inhibition, the $K_i$ value for undecanesulfonate was calculated to be 72 μM.

**FIG. 7.** Lauric acid, but not undecanesulfonic acid, can diffuse across the liposomal membranes. Inverse SPQ fluorescence (1/F) is plotted versus time in liposomes lacking UCP and containing internal medium for H$^+$ transport (see "Experimental Procedures"). A decrease in 1/F indicates protonation of TES anion and, hence, delivery of protons across the liposomal membrane. Arrows indicate additions of 25 μM sodium laurate (laurate) or 50 μM sodium undecanesulfonate (undecanesulfonate).

**TABLE I**

| Anion       | $V_{max}$/nmol/mg UCP-min | $K_m$/μM | Limiting permeability |
|-------------|---------------------------|----------|-----------------------|
| Cl$^-$      | 9,000                     | 1.4 $\times$ 10$^{-1}$ | 6.4 $\times$ 10$^{-5}$ |
| Hexanesulfonate | 23,500                 | 1.2 $\times$ 10$^{-2}$ | 2.0 $\times$ 10$^{-3}$ |
| Undecanesulfonate | 37,600                | 1.2 $\times$ 10$^{-2}$ | 3.1                 |
| Laurate     | 22,000                     | 8.0 $\times$ 10$^{-6}$ | 2.8                 |
| Oleate      | 16,000                     | 5.0 $\times$ 10$^{-6}$ | 3.2                 |

**DISCUSSION**

The Mechanism by Which Fatty Acids Induce UCP-mediated Proton Transport—Our understanding of UCP transport mechanism is built on foundations laid by Nicholls and co-workers (1), beginning with the discovery that UCP conducts anions of strong acids (18). Nicholls and Locke (20) pointed out that anion transport is without physiological significance and concluded that Cl$^-$ and other anions are accidental substrates of UCP. In our view, it is this very aspect of anion transport through UCP that holds the key to the mystery of UCP-mediated H$^+$ transport. This view was strengthened by our finding that short chain (C1-C8) alkylsulfonates are transported by UCP and that their flux increases with alkyl chain length (5). These results are now extended to long-chain alkylsulfonates: (i) undecanesulfonate is transported as the anion by UCP with $K_m$ values of 10–15 μM (Fig. 3); (ii) laurate and undecanesulfonate are competitive inhibitors of Cl$^-$ transport (Fig. 5); and (iii) undecanesulfonate is a competitive inhibitor of laurate-induced H$^+$ transport (Fig. 6). These and other results strongly
support the hypothesis that anions are transported through the FA docking site in UCP (5, 7, 8).

How do FAs activate UCP-mediated proton transport? This is the principal unsolved question surrounding the molecular basis of UCP mechanism. Winkler and Klingenberg (9) propose that multiple FAs bind at sites within the UCP proton channel, thereby providing local acceptor/donor groups that facilitate H⁺ transport (the Buffering Model). Based on work showing FA-induced H⁺ transport via the ADP/ATP carrier, Skulachev (10) proposes that UCP transports FA anions directly, with protons transported via nonionic diffusion of the protonated FA (the Protonophoretic Model). Our experiments comparing the behavior of laurate and its close analogue, undecanesulfonate, provide a plausible means of distinguishing between these models.

Most features of undecanesulfonate behavior are consistent with either model. Interpreted according to the Buffering Model, the results imply that undecanesulfonate competes successfully with laurate in binding to the FA-binding sites within the UCP proton channel, thereby inhibiting laurate-induced H⁺ transport (Fig. 6). Because it is a strong acid, it provides no buffering in the channel and therefore cannot support UCP-mediated H⁺ transport (Fig. 2). Interpreted according to the Protonophoretic Model, undecanesulfonate, like other anions, is transported through the FA anion channel of UCP. It does not support UCP-mediated H⁺ transport because it is incapable of nonionic delivery of protons across the bilayer (Fig. 7).

A crucial distinction between the two models is that FA binding and transport is stochiometrically linked to H⁺ transport in the Protonophoretic Model, whereas FA remain bound to the protein in the Buffering Model and do not participate stoichiometrically in H⁺ transport. In this respect, our data support the Protonophoretic Model on both qualitative and quantitative grounds: undecanesulfonate anion is transported by UCP. We are unaware of any physicochemical mechanism to explain why laurate anion should remain bound without also undergoing transport. Furthermore, undecanesulfonate, as a stand-in for laurate anion, is transported with V_{max} and K_m values that are fully sufficient to account for laurate-induced H⁺ transport via a stoichiometric, protonophoretic mechanism (Table I). Applying Occam's razor, we infer that FA catalyze UCP-mediated proton conductance because the FA anion is transported by UCP and the protonated FA is rapidly transported across the lipid bilayer by nonionic diffusion (Fig. 8).

This proton cycling mechanism is entirely analogous to uncoupling by weak acid protonophores. It differs in that a specific protein is required in order to provide a conductance pathway for back-diffusion of the FA anion.

The UCP-catalyzed Protonophoretic Cycle—The protonophoretic FA cycle consists of the following steps, as diagrammed in Fig. 8.

(i) FA anion partitions in the lipid bilayer with its head group at the level of the acylglycerol linkages and below the surface of the phospholipid head groups. This shielded location, driven by the free energy of partitioning of the alkyl chain, is responsible for the long-standing observation that the pKₐ values of FAs in membranes are 3 to 4 units higher than their values in solution (22). Despite high electrical gradients, there is no significant flux of FA anion, because the bilayer energy barrier is too high.

(ii) The FA anion diffuses laterally in the bilayer to reach the protein. UCP may contain a weak binding site to concentrate the anion in the conductance pathway. If so, this site must also be partly buried, because kinetic studies show that it is shielded from the bulk aqueous phase (6).

(iii) The energy barrier to anion transport is lowered by a weak binding site located about halfway through the UCP transport pathway. (The existence of this energy well was deduced from the dependence of UCP-mediated Cl⁻ flux on Δψ (8)). The anionic head group is driven to this energy well by the electric field created by redox-linked proton ejection. Given the preference for hydrophobic substrates, it seems likely that all or part of the conductance pathway lies on the outer surface of the protein, at the lipid-protein interface.

(iv) The anionic carboxyl group moves to the other side of the membrane by a flip-flop mechanism such as occurs during nonionic transmembrane diffusion of protonated FAs (23). The FA anion then diffuses laterally away from the conductance pathway.

(v) The FA is protonated and rapidly flip-flops again, delivering protons by nonionic diffusion to the mitochondrial matrix and completing the cycle.

Properties of the UCP Anion Transport Pathway—V_{max} values reflect the rate constant for crossing the second energy barrier as anions leave the saturated energy well and move to the other side. Cl⁻ exhibits the lowest V_{max} among the anions in Table I, suggesting that the alkyl chain facilitates transport along the hydrophobic protein-lipid interface. The limiting permeabilities (V_{max}/K_m), which equal J_{anion} as [anion] approaches zero, reflect the rate constants for passage over the first energy barrier and also contain the coefficients of anion partitioning into the pathway. The limiting permeabilities of undecanesulfonate, laurate, and oleate are very nearly the same (Table I). The lower value for hexanesulfonate can be rationalized by its lower partition coefficient. Small polar an-
ions, such as halides (5, 18), acetate (7), and nitrate (6, 18), cannot partition into the hydrocarbon at all and must gain additional thermal energy to reach the same starting location as hydrophobic anions. This is acquired through normal thermal bombardment of the total membrane surface. Thus, their limiting permeabilities are very low, and their \( K_m \) values are correspondingly high.

Bioenergetic Aspects of the Fatty Acid Protonophore Hypothesis—In mitochondria containing 10% UCP protein, the \( V_{\text{max}} \) for laurate (Table I) translates to about 2000 nmol/(mg of mitochondrial protein \( \times \) min), roughly equal to the maximum rate of proton ejection by the redox chain. This is not unexpected, because mitochondrial transporters are normally synthesized in quantities more than sufficient to carry out their tasks. The maximum turnover number estimated from these data is about 20 \( s^{-1} \). This rather sluggish rate emphasizes another physiological control mechanism for UCP. When the need for thermogenesis is great, mammals synthesize more UCP, to the extent that UCP levels reach 15% of mitochondrial protein (24, 25).

It should be emphasized that long chain FAs that reach the matrix by nonionic diffusion cannot enter the \( \beta \)-oxidation pathway, because the matrix doesn’t contain long chain acyl-CoA synthetase (21). Instead, acylcarnitine is transported to the matrix where it is activated by carnitine acyltransferase II. Since FA rapidly equilibrate across the inner membrane, it may be useful to view carnitine/acylcarnitine translocase as being required for channeling of long chain FAs into the \( \beta \)-oxidation pathway.

Summary—The uncoupling protein of brown adipose tissue mitochondria mediates electrophoretic transport of anions and protons, and proton transport requires fatty acids for activation. Transported anions and FAs interact with a common site on UCP which we have called the FA docking site (6, 8). The differential effects of laurate and its analogue, undecanesulfonate, support the hypothesis that the protonophoretic mechanism of UCP relies on FA anion transport. That is, UCP contains a conductance pathway for anions and does not transport protons. We propose that this pathway lies in the protein-lipid interface.

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