

Mechanism of uncoupling protein action

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Abstract

Two competing models of uncoupling protein (UCP) transport mechanism agree that fatty acids (FAs) are obligatory for uncoupling, but they disagree about which ion is transported. In Klingenberg's model, UCPs conduct protons. In Garlid's model, UCPs conduct anions, like all members of this gene family. In the latter model, UCP transports the anionic FA head group from one side of the membrane to the other, and the cycle is completed by rapid flip-flop of protonated FAs across the bilayer. The head groups of the FA analogues, long-chain alkylsulphonates, are translocated by UCP, but they cannot induce uncoupling, because these strong acids cannot be protonated for the flip-flop part of the cycle. We have overcome this limitation by ion-pair transport of undecanesulphonate with propranolol, which causes the sulphonate to deliver protons across the membrane as if it were an FA. Full GDP-sensitive uncoupling is seen in the presence of propranolol and undecanesulphonate. This result confirms that the mechanism of UCP uncoupling requires transport of the anionic FA head group by UCP and that the proton transport occurs via the bilayer and not via UCP.

Introduction

Uncoupling protein (UCP) 1 has been extensively studied during the last 30 years, and it is remarkable that the uncoupling mechanism and even the identity of the ionic species transported continue to be a source of controversy. This paper will review the available evidence relating to the two major hypotheses that have been advanced to explain the mechanism of fatty acid (FA)-induced uncoupling.

Key words: carrier, fatty acids, mitochondria, proton transport, UCP.

Abbreviations used: BAT, brown adipose tissue; C₁₁-sulphonate, undecanesulphonate; FA, fatty acid; UCP, uncoupling protein.

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The FA buffer model of uncoupling by UCP

In this model, proposed by Klingenberg and co-workers [1–3], intramembrane FAs insert their head groups into the H⁺ transport pathway and provide buffering sites to assist H⁺ translocation through UCP. The hypothesis is superficially plausible, but eventually reaches a logical paradox. FAs penetrate all along the protein axis and from either side of the protein. The FAs give up their protons at these sites, so the carboxylate head group is now found along the length of UCP [3]. Thus, this model requires substantial transport of the FA anion head group along UCP but insists that the head group is not transported all the way to the other side, despite the presence of a very large electrical driving force.

This hypothesis ignores anion transport, although the UCPs have long been known to transport anions [4,5]. The model assumes that UCPs transport protons; however, we have now demonstrated that UCP-mediated uncoupling does not involve proton translocation by UCP, but rather requires H⁺ translocation in the bilayer [6].

Our primary criticism of the model derives from the behaviour of alkylsulphonates, which are identical to FAs except for their head groups. Inasmuch as the anionic head group of long-chain alkylsulphonates is transported across the membrane by UCP1, there is no physicochemical basis for exclusion of the FA anionic head group from this pathway [7].

The FA Protonophore Model of uncoupling by UCP

In this model, proposed by Garlid and co-workers [6–9] and shown in Figure 1, the UCPs translocate the anionic head groups of FAs and alkylsulphonates. The head group is driven from one side of the membrane to the other by the electric field generated by electron transport. When the FA carboxylate reaches the other side, it picks up a proton and rapidly flip-flops back, to release the proton. The UCPs thus catalyse a regulated

protonophoretic cycle, leading to uncoupling of oxidative phosphorylation. This model, in which uncoupling is due to the known anion-transport function of UCP, conforms to the transport function of the other members of the gene family of mitochondrial anion carriers [10].

Evidence favouring the FA Protonophore Model derives in large part from the discovery that alkylsulphonates ranging in alkyl chain length between C_1 and C_{16} are transported by UCP1 [6,7,11]. Both the V_{max} and the apparent affinity ($1/K_m$) for transport increase with increasing chain length. Alkylsulphonates are competitive inhibitors of UCP-mediated Cl^- transport, and the apparent affinity ($1/K_i$) for inhibition also increases with hydrophobicity [11]. This implies that the sulphonates interact with a buried hydrophobic site on UCP1 and that access to this site is favoured by partitioning in the membrane [11].

Long-chain alkylsulphonates are particularly interesting, because they are identical to FAs except for the head groups, and they are ideal probes of the UCP transport mechanism for two reasons. First, undecanesulphonate (C_{11} -sulphonate) closely resembles its analogue, laurate, in its interactions with UCP1: C_{11} -sulphonate is trans-

ported by UCP1 with K_m and V_{max} values similar to those obtained for laurate-dependent H^+ transport [7]; C_{11} -sulphonate and laurate-dependent H^+ transport are inhibited by GDP with the same K_i [6]; and C_{11} -sulphonate and laurate are mutually competitive inhibitors [6]. A second reason is that the alkylsulphonates probe only the UCP-mediated half of the uncoupling cycle. Thus, C_{11} -sulphonate cannot support UCP-mediated H^+ flux, because it cannot flip-flop with protons across the bilayer membrane, due to its very low pK_a [7]. Therefore, a primary underpinning of the FA Protonophore Model is that laurate and C_{11} -sulphonate share a common anion-transport pathway in UCP, but that C_{11} -sulphonate cannot complete the cycle, because it cannot flip-flop with protons across the membrane.

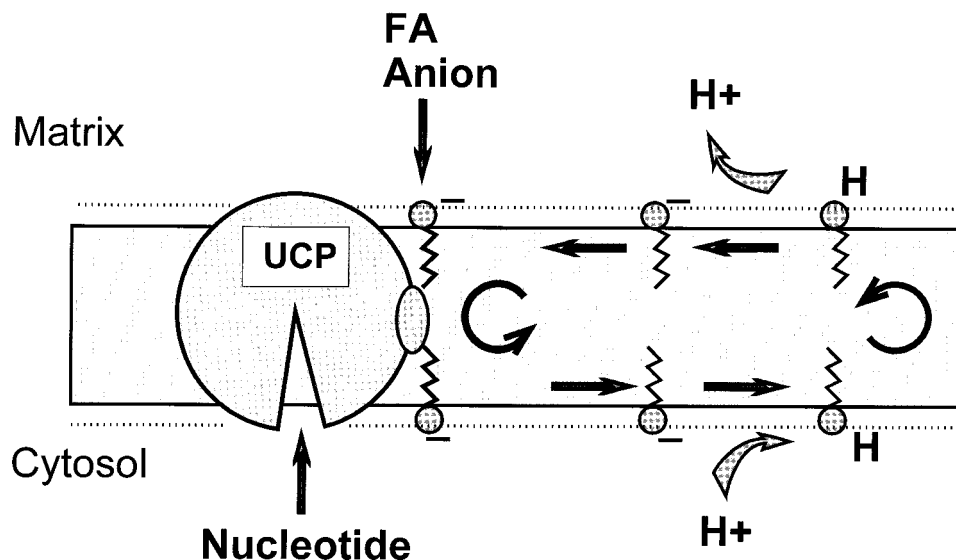
Criticisms of the FA Protonophore Model

Klingenberg and co-workers [1–3] have raised a number of objections to the model that are addressed in detail elsewhere [6]. Three arguments deal with alkylsulphonates, as follows. (a)

Figure 1

The FA Protonophore Model of UCP transport mechanism

FA anion diffuses laterally within the membrane to reach a subsurface binding site on UCP. The membrane potential drives the carboxylate to an energy well located halfway through the UCP transport pathway and then over another energy barrier to the other side of the membrane. The FA anion diffuses laterally away from UCP, where it is protonated. Protonated FA diffuses rapidly back across the membrane to deliver protons electroneutrally to the matrix by a spontaneous flip-flop mechanism, completing the cycle. The UCP-catalysed protonophoretic cycle dissipates redox energy and produces heat. Reproduced, with permission, from [14]. © The American Society for Biochemistry & Molecular Biology.



C_{11} -sulphonate inhibition of laurate-dependent H^+ flux is due to competitive removal of FAs from the membrane by C_{11} -sulphonate [1,2]. We have shown directly that this is not the case, nor should such an effect be expected [6]. (b) C_{11} -sulphonate is not transported by UCP1 but is driven across the membrane in a ternary complex with valinomycin and K^+ [1,2]. To the contrary, GDP-sensitive C_{11} -sulphonate transport via UCP1 can be driven by a proton gradient and CCCP (carbonyl cyanide *m*-chlorophenylhydrazine), which obviously cannot form ternary complexes with sulphonates [6]. (c) The GDP-sensitivity of alkylsulphonate transport is low and decreases with increasing chain length [3]. This statement is incorrect for long-chain sulphonates. K_i values for GDP inhibition of laurate, Cl^- , and C_{11} -sulphonate transport are the same, about $15 \mu M$ at pH 7.2 [6].

Echtay et al. [12,13] have recently confirmed the first report showing that UCP2 and UCP3 catalyse FA-dependent H^+ transport [14]. In that paper, we also demonstrated C_{11} -sulphonate transport by these UCPs, and suggested that they also uncouple by the FA Protonophore Mechanism

[14]. Klingenberg and Echtay [3] now state that the H^+ flux that we reported is “only a few percent of that measured with UCP1”. This is incorrect. It is clearly stated in [14] that the V_{max} values for UCP2 and UCP3 were $10\text{--}30 \mu mol/min$ per mg of protein, approximately the same as the V_{max} values observed with UCP1. Klingenberg’s laboratory routinely reports excessively high rates of H^+ transport. We agree entirely with their admission [1] that these rates are inconsistent with what is known about UCP1 activity in brown adipose tissue (BAT) mitochondria [9].

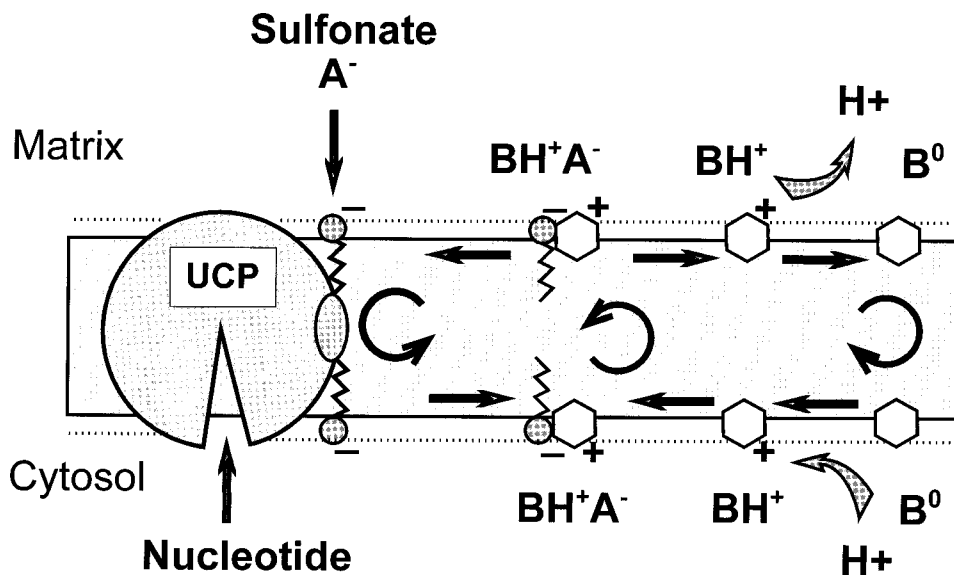
Evidence that protons are not transported by UCP

According to our hypothesis, C_{11} -sulphonate does not support UCP-mediated uncoupling because it cannot flip-flop with protons across the lipid bilayer [7]. An important prediction of the hypothesis, therefore, is that removal of this restriction would lead to C_{11} -sulphonate-dependent uncoupling in BAT mitochondria and in liposomes reconstituted with UCP1. Amphiphilic

Figure 2

The mechanism of C_{11} -sulphonate uncoupling via ion-pair transport with propranolol

The sulphonate head group has a very low pK_a , so alkylsulphonates cannot undergo spontaneous flip-flop with protons. This limitation can be overcome by means of ion-pair transport, as shown. Alkylsulphonate, A^- , forms an ion pair with the protonated amine, BH^+ , and the electroneutral complex diffuses across the bilayer. Upon dissociation, the amine loses its proton, and the free base, B^0 , diffuses back across the membrane. This part of the cycle occurs in the bilayer and causes alkylsulphonate to distribute and deliver protons as if it were an FA. This step is electroneutral and does not lead to uncoupling. When alkylsulphonate is transported electrophoretically via UCP, a protonophoretic cycle will be set up. Thus if UCP only transports anions, then alkylsulphonates should behave like fatty acids in the presence of an ion-pairing amine. This is indeed the case, both in proteoliposomes and in BAT mitochondria [6].



amines support ion-pair transport of SCN^- and H^+ across the mitochondrial inner membrane [15], and we recently found that they also support ion-pair transport of C_{11} -sulphonate in liposomes and BAT mitochondria [6].

Ion-pair transport causes electroneutral equilibration of H^+/C_{11} -sulphonate across the membrane, as shown in the model in Figure 2, enabling C_{11} -sulphonate to behave as a pseudo-FA. When valinomycin is added for charge compensation, C_{11} -sulphonate plus propranolol now supports GDP-sensitive, UCP-mediated uncoupling [6]. Ion-pair transport converts the C_{11} -sulphonate + UCP system from a pure anion-transport system to an electrophoretic H^+ -transport system, thus confirming this important prediction of the FA Protonophore Model. We have also shown that C_{11} -sulphonate + propranolol, but not C_{11} -sulphonate alone, causes GDP-sensitive uncoupling of BAT mitochondria [6].

These experiments show that proton transport across the *bilayer* is the only factor preventing UCP-mediated uncoupling by alkylsulphonates, as predicted by the FA Protonophore Model. Thus, we conclude that UCP does not transport protons, but rather transports the FA anion, and that the protons are translocated by spontaneous FA flip-flop in the bilayer.

Our work was supported by National Institutes of Health grant DK56273.

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Received 31 May 2001

Uncoupling protein, H^+ transport and regulation

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Abstract

The biochemical functions of uncoupling proteins (UCPs) are discussed with the view of UCP1 as a paradigm. In contrast with UCP1, the heterologous expression of UCP3 in yeast is found to result primarily in extra-mitochondrial deposits and thus is unsuitable for studying UCP3 function. On expression in *Escherichia coli* inclusion bodies, UCPs extracted and incorporated into vesicles showed no H^+ transport, only Cl^- trans-

port. Only after addition of coenzyme Q was fully nucleotide-sensitive high- H^+ transport reconstituted, with UCP1 as well as with UCP2 and UCP3. The newly discovered cofactor role of coenzyme Q in H^+ transport is proposed to imply co-operation with fatty acids for the injection of H^+ into the UCP channel.

Introduction

Uncoupling proteins (UCPs) are suggested by their name to uncouple oxidative phosphorylation [1]. Whereas there was little doubt about the uncoupling function of UCP1 from brown adipose tissue, for other UCPs (e.g. UCP2–5) the uncoupling and the synonymous H^+ -transport func-

Key words: coenzyme Q, reconstitution, yeast/*Escherichia coli* expression.

Abbreviations used: UCP, uncoupling protein; CoQ, coenzyme Q.

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