The binding of dicyclohexylcarbodiimide to uncoupling protein in brown adipose tissue mitochondria

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1. INTRODUCTION

The generation of heat in brown adipose tissue has been related to the uncoupling of respiration in their mitochondria (reviews [1,2]). The uncoupling in these mitochondria is achieved by a high ion conductance of the mitochondrial membrane and is specifically controlled by exogenous purine nucleotides [3–7]. The binding site for these nucleotides was identified as a 32 000 M_r protein that is present in considerable amount only in brown adipose tissue mitochondria [8–11]. It is proposed that this protein (so called GDP-binding or uncoupling protein) is directly responsible for the physiological uncoupling of brown adipose tissue mitochondria.

Here, we report that *N,N'*-dicyclohexylcarbodiimide a well-established inhibitor or proton-pumping activity of several membrane enzymes [12–16], binds rather specifically to the uncoupling protein in brown adipose tissue mitochondria. In addition, it inhibits the high chloride permeability of brown adipose tissue mitochondrial membrane. We propose that the 32 000 M_r uncoupling protein may function in mitochondrial membrane of brown adipose tissue similarly to other proton-translocating DCCD binding proteins.

Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; BAT, brown adipose tissue; SDS, sodium dodecyl sulfate; TES, *N*-Tris-(hydroxymethyl)methyl-2-aminoethanesulfonic acid

2. MATERIALS AND METHODS

[^{14}C]DCCD (spec. act. 32 mCi/mmol) was synthesized as in [17]. Labeled and unlabeled DCCD were used as methanolic solutions and appropriate controls were run with methanol alone. Mitochondria from different tissues were isolated according to published procedures [18,19]. ADP/ATP translocator protein was isolated as in [20]. ^{14}C-Labeled M_r standards were from The Radiochemical Centre, Amersham.

Determination of the amounts of [^{14}C]DCCD bound to mitochondria and the evaluation of maximum binding capacity was performed as in [21,22]. Uncoupling protein was isolated from [^{14}C]DCCD-labeled brown adipose tissue mitochondria (8–10 mg mitochondrial protein) by scaling down the procedure of [11]. After the chromatography step [11] the fractions with the highest protein-bound radioactivity were electrophoresed on SDS–polyacrylamide slab gels and autoradiographed as in [23]. The swelling of brown adipose tissue mitochondria was followed spectrophotometrically at 540 nm after addition of the mitochondria to 0.1 M KCl or K-acetate with 5 μM rotenone, 0.5 μM valinomycin and 5 mM TES (pH 7.2) as in [3]. Publised methods were used for sample solubilization, electrophoresis in SDS–polyacrylamide 12–20% gradient gels [24] and fluorography [23].

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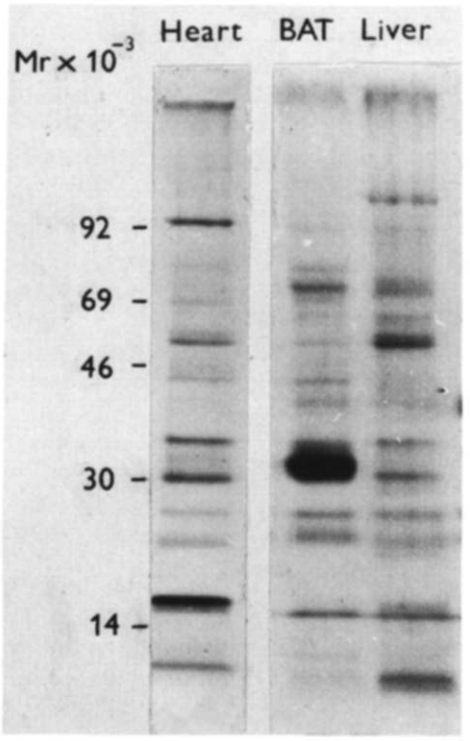
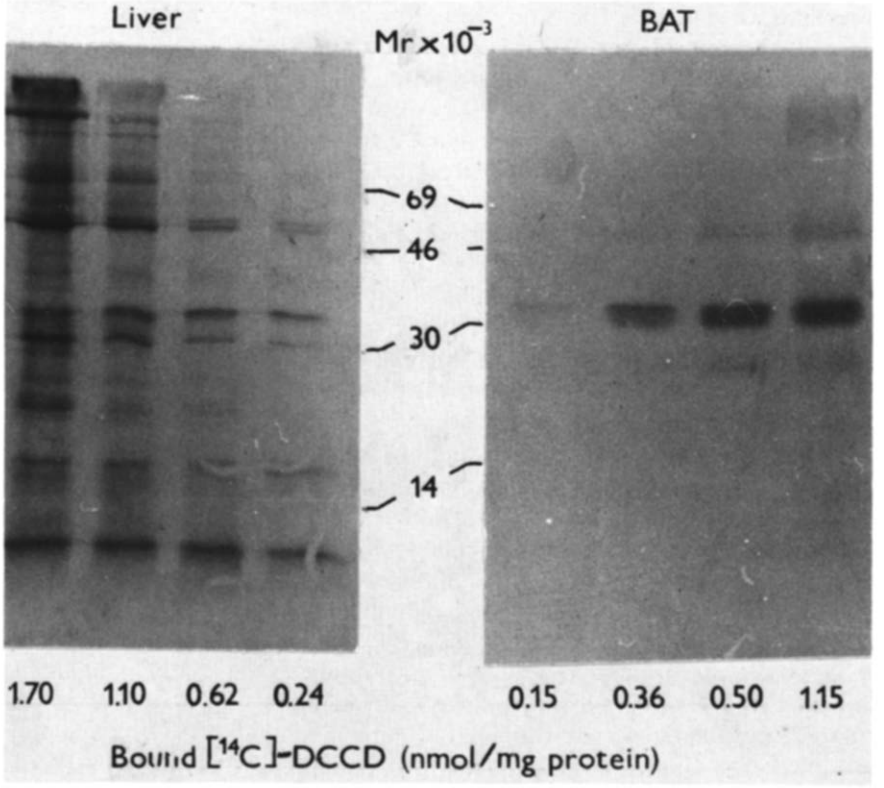


Fig.1. Binding of [^{14}C]DCCD to mitochondria isolated from different tissues. Isolated rat heart, liver and BAT mitochondria (5 mg/ml) were incubated for 90 min at 15°C in a medium containing 0.25 M sucrose, 3.4 mM Tris–HCl, 1 mM EGTA (pH 7.4) and 350 nmol [^{14}C]DCCD/mg protein. Aliquots from the labeled mitochondria were centrifuged, washed once with the same solution without DCCD then twice with 90% acetone, solubilized and electrophoresed in SDS–polyacrylamide gels as in section 2. Radioactivity distribution was detected by fluorography. The positions of the ^{14}C-labeled M_r standards in the gel are indicated.

Fig.2. Binding of [^{14}C]DCCD to rat liver and hamster BAT mitochondrial proteins. Isolated rat liver or hamster BAT mitochondria were incubated with different concentrations of [^{14}C]DCCD (5–100 nmol/mg protein) under the conditions identical with those in fig.1. Aliquots were also taken to determine the amount of [^{14}C]DCCD bound/mg total mitochondrial protein (shown under the respective lines).



3. RESULTS AND DISCUSSION

3.1. *Tissue specific pattern of labeling of mitochondrial proteins by [^{14}C]DCCD*

Upon incubation of mitochondria from different rat organs with high concentration of [^{14}C]DCCD a marked difference in the pattern of labeled peptides was observed (fig.1). In contrast to heart or liver mitochondria in brown adipose tissue mitochondria the main portion of the bound [^{14}C]DCCD (> 75%) was recovered after SDS–polyacrylamide gel electrophoresis in a single radioactive band. The electrophoretic mobility of this DCCD-binding protein(s) in brown adipose tissue mitochondria corresponds to M_r 32 000.

The binding of [^{14}C]DCCD to brown adipose tissue mitochandria differs both quantitatively and qualitatively from the binding to rat heart or liver mitochondria. In agreement with [22] the incubation of brown adipose tissue mitochondria with < 5 nmol [^{14}C]DCCD/mg protein resulted in a weak labeling of mitochondrial proteins. With higher [^{14}C]DCCD levels a gradual and almost selective increase of the radioactivity in 32 000 M_r region was observed (fig.2). Under identical conditions of labeling, liver (fig.2) as well as heart (not shown) mitochondria exhibited increased number of different [^{14}C]DCCD labeled peptides. Since most of the [^{14}C]DCCD label in brown adipose tissue mitochondria is concentrated in a 32 000 M_r region the maximum binding capacity of 3.5 nmol DCCD/mg protein of brown adipose tissue mitochondria indicates a high content of this protein(s) in the mitochondrial membrane.

3.2. *The isolation of uncoupling protein from [^{14}C]DCCD labeled brown adipose tissue mitochondria*

The high content of DCCD binding 32 000 M_r protein(s) in brown adipose tissue mitochondria as well as its apparent absence in liver and heart mitochondria suggest that it may be identical with the uncoupling protein of these organelles. Accordingly, a 32 000 M_r labeled peptide was isolated from [^{14}C]DCCD-labeled mitochondria (fig.3, lines 2,3,5) using a purification procedure for uncoupling protein. The isolated protein is clearly different from ADP/ATP translocator protein (fig.3, line 1) a possible contaminant of the isolated uncoupling protein [11]. In addition, no such labeled peptide could be isolated from liver or heart mitochondria using the same procedure. The above results provide strong evidence that the 32 000 M_r DCCD-binding protein of brown adipose tissue mitochondria is identical with the uncoupling protein.

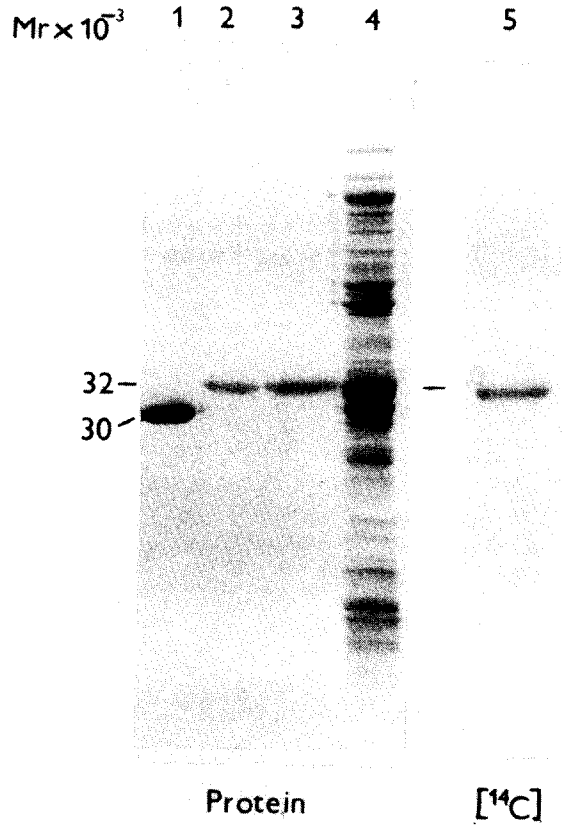


Fig.3. Electrophoretic separation of uncoupling protein isolated from [^{14}C]DCCD-labeled brown adipose tissue mitochondria. Uncoupling protein was isolated from 7.6 mg hamster brown adipose tissue mitochondria labeled with [^{14}C]DCCD (0.6 nmol [^{14}C]DCCD bound/mg protein) at conditions similar to those in fig.1: Lanes (2,3,5) isolated uncoupling protein; (1) isolated ADP/ATP translocator protein; (4) hamster brown adipose tissue mitochondria.

3.3. *The inhibition by DCCD of the high ion permeability of brown adipose tissue mitochondria*

On the basis of the above results it can be expected that DCCD could affect the specific function of

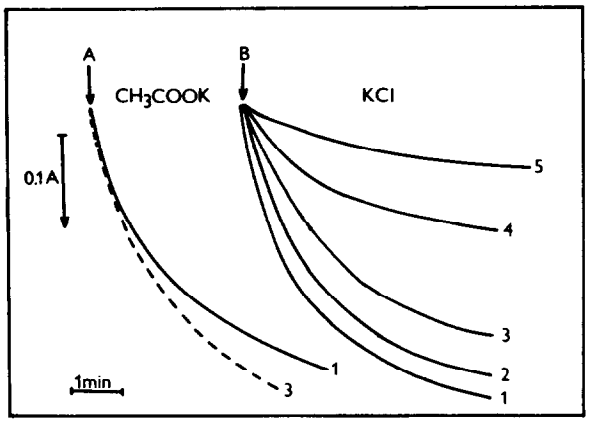


Fig.4. Effect of DCCD on the swelling of brown adipose tissue mitochondria in potassium acetate and KCl solutions. Hamster brown adipose tissue mitochondria (0.4 mg protein) were added tot 1 ml medium containing in (A) 0.1 M K-acetate, or in (B) 0.1 M KCl, both with 5 μM rotenone, 0.5 μM valinomycin and 5 mM K-TES (pH 7.2). Controls (1) treated with 2% methanol are compared with (2,3,4) mitochondria treated for 2 h at 0°C with 50, 100 and 500 nmol DCCD/mg protein, respectively. The effect of 50 nmol GDP on the swelling in KCl solution is also shown (5).

uncoupling protein in brown adipose tissue mitochondrial membrane. Accordingly, the preincubation of brown adipose tissue mitochondria with DCCD has an inhibitory effect on the high chloride permeability of the mitochondrial membrane as detected by the swelling in KCl solutions (fig.4). The swelling in potassium acetate was not significantly affected by DCCD. This indicated that the inhibitor affects the purine nucleotide regulated anion transport into brown adipose tissue mitochondria.

These results provide strong evidence that the protein responsible for the uncoupling of brown adipose tissue mitochondria regulated by purine nucleotide is a dominant DCCD-binding protein in these organelles. Inhibition of purine nucleotide-sensitive ion transport by DCCD most probably results from the binding of DCCD to the uncoupling protein. Thus the possibility exists that the uncoupling protein in brown adipose tissue mitochondria is structurally and functionally analogous to other proton-translocating DCCD binding proteins.

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