PARTIAL PURIFICATION BY GUANOSINE-5'-DIPHOSPHATE–AGAROSE AFFINITY CHROMATOGRAPHY OF THE 32 000 MOLECULAR WEIGHT POLYPEPTIDE FROM MITOCHONDRIA OF BROWN ADIPOSE TISSUE

D. RICQUIER, C. GERVAIS, J. C. KADER+ and Ph. HEMON

Laboratoire de Physiologie comparée and +*Laboratoire de Physiologie cellulaire (ERA 323), Université P. et M. Curie, 4, Place Jussieu, 75230 Paris Cedex 05, France*

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1. Introduction

It has been demonstrated that in hamsters [1], purine nucleotides, which are able to restore respiratory control and energy conservation in isolated brown adipose mitochondria [2–6] are associated with a high affinity binding site which is a 32 000 mol. wt membrane protein; the binding of GDP or ADP to these mitochondria has been well characterized [7–10].

Prior to the data reported [1], a polypeptide of 32 000 mol. wt has been reported to increase strikingly in membranes of brown adipose tissue mitochondria during cold adaptation of rats [9,11].

The relative amount of the 32 000 mol. wt polypeptide, referred to in the text as the 32 000 component, has been studied during development of young rats and after cold exposure. The more important part of this work deals with the partial purification of this polypeptide using GDP–agarose affinity chromatography of solubilized (Triton X-100, 0.25%) mitochondrial membranes.

2. Materials and methods

Mitochondria were isolated from intercapsular brown adipose tissue or from liver of rats (Sprague-Dawley strain) of different ages and kept at 25°C (control animals) or at 6°C (cold-exposed animals) [12]. Proteins were determined using a modification of the Lowry method [13], with bovine serum albumin used as a standard.

Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed as in [11] with addition of 2 M urea to electrophoresis buffer.

Affinity chromatography was performed using GDP–agarose (Sigma). Mitochondria were treated with 1 M KCl, left at 0°C for 15 min, then centrifuged at 13 000 × *g* for 15 min. Only 10% of the pellet proteins could be solubilized, into 1 ml buffer (0.1 M KCl–10 mM Tris (pH 7.2)–1 mM EDTA–0.25% Triton X-100). After 15 min at 0°C the mixture was sedimented at 20 000 × *g* for 20 min. The supernatant was then passed through GDP–agarose column (6 mm i.d. 8 cm length) with buffer at a 4.5 ml/h flow rate and at 3°C. Elution of the GDP-bound proteins was made with the same buffer supplemented with 8 mM GDP. The volume of each eluted fraction was 0.5 ml.

3. Results and discussion

3.1. *Developmental changes*

The large amount of the 32 000 component observed in day 10 rats (see fig.1) accounts for the observation [14,15] of an increased GDP-binding capacity of isolated mitochondria from young rats. Similarly, a 4 day exposure to cold provokes an increase in the amount of the polypeptide, although an increase after a shorter time has been reported [9]. These data demonstrate that in a high thermogenic state, brown adipose tissue contains an increased quantity of 32 000 mol. wt GDP binding site, similar data having been reported with guinea-pigs [1,8].

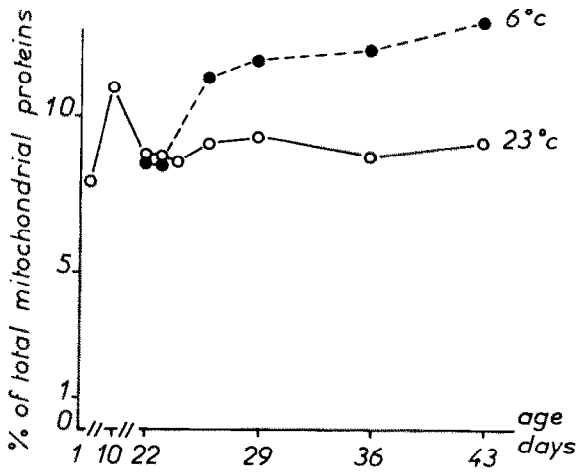

Fig.1. Relative amount of the 32 000 polypeptide in mitochondrial proteins of rat brown adipose tissue. Effect of age and cold exposure. The amount was measured after separation of polypeptide by SDS–PAGE (—○—) control animals bred at 25°C. (- - -●- - -) 6°C exposed rats since the age of 22 days up to the age of 43 days (this work had been done with Wistar animals).

3.2. *Comparison between liver and brown fat mitochondria*

When a comparison of the polypeptide composition of rat liver and brown adipose tissue mitochondria was made, several differences could be found (see also [16]) (fig.2). In contrast to the observation made on brown fat mitochondria, no effect of cold exposure upon liver mitochondria was seen. Therefore the 32 000 polypeptide seems to be characteristic of brown adipose tissue mitochondria. Moreover this protein is the binding site of purine nucleotides [1] and this GDP binding is not apparent in liver mitochondria [6–8].

3.3. *Affinity chromatography of solubilized mitochondrial proteins*

When GDP (8 mM) was added to the buffer passing through affinity column, a small peak of protein was rapidly eluted (fig.3B). Analysis of the components of this peak showed 2 minor bands and 2 major bands (103 000 and 32 000 mol. wt) (fig.3C). We do not know if there is any relationship between the 103 000 mol. wt component and a polypeptide of 96 000 mol. wt reported [9] to decrease when the 32 000 mol. wt polypeptide increased. It must be pointed out that exactly similar results have been obtained in 8 separate experiments and led to the partial purification of the 32 000 mol. wt polypeptide.

Table 1 gives data obtained using different conditions for solubilization: the purification was not modified by atractyloside and was sensitive to changes in pH and to addition of $MgCl_2$; these results could be explained by data concerning the binding and the effect of GDP in isolated mitochondria [6,7,17]. Moreover elution of the purified fraction could be obtained either with GDP, GTP, ADP or ATP. A similar elution was not obtained with CDP, which is consistent with experiments showing that pyrimidine nucleotides were ineffective in restoring respiratory control in isolated mitochondria [6,18]. All these results are in agreement with the idea that the puri-

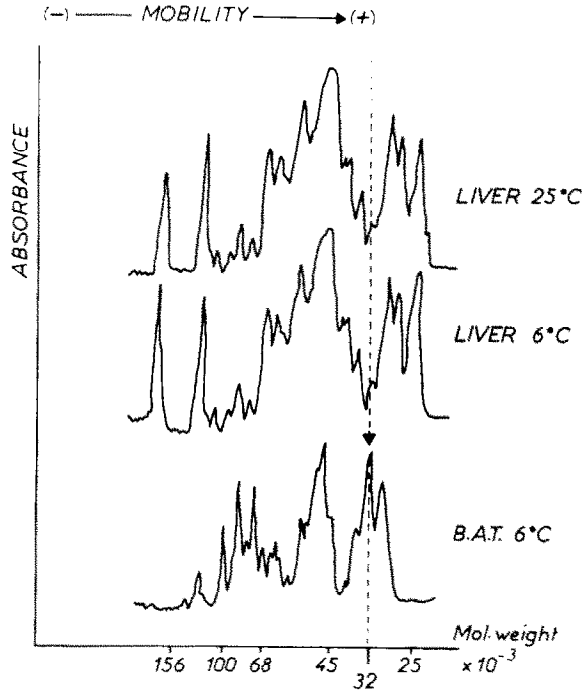

Fig.2. SDS–PAGE (7.5% acrylamide) of mitochondrial proteins from liver and brown adipose tissue. Upper traces: polypeptides of liver mitochondria from control (25°C) or cold-adapted rats (6°C). Lower trace: polypeptides of brown fat mitochondria from the same cold-adapted rats.

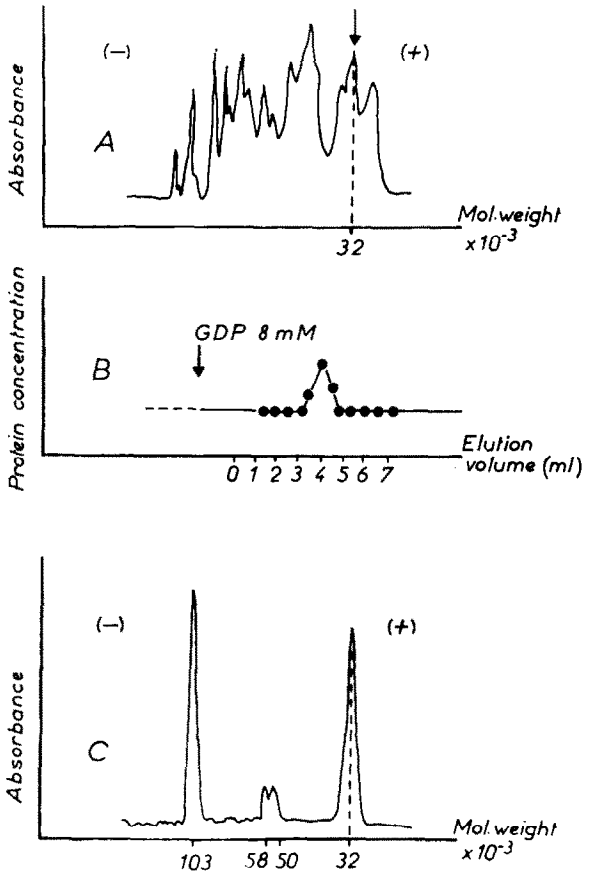

Fig.3. Partial purification of the GDP-binding site of rat brown adipose tissue mitochondria using affinity chromatography (cold-adapted rats). (A) SDS–PAGE of solubilized mitochondrial proteins passed through affinity column. (B) Elution of proteins bound to agarose affinity gel by added 8 mM GDP. (C) SDS–PAGE of eluted proteins from GDP–agarose affinity-column.

fied 32 000 fraction is the purine nucleotide binding site of brown adipose tissue mitochondria. Moreover some preliminary experiments suggested a glycoprotein nature for this polypeptide. This partial purification of the 32 000 mol. wt protein is in good agreement with results [1] where a covalent linkage of azido-nucleotides to this mitochondrial component was obtained. This purification of the polypeptide is a good indication of its affinity for GDP and of its identity with the regulatory GDP binding site characteristic of these mitochondria.

Table 1
Effect of different experimental conditions upon the partial purification of the 32 000 mol. wt protein

Solubilization conditions		Purification of the polypeptide
Triton X-100,	0.25%	+
	0.10%	–
	0.15%	–
Tween-80	0.25%	+
Deoxycholate	0.50%	–
Lubrol	0.20%	–
Triton X-100	+2 mM $MgCl_2$	–
	+Atractyloside	+
	at pH 7.9	–
Sonication		–
Triton X-100	with liver mitochondria	–

+ good recovery of the protein; – no recovery of the protein

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