MITOCHONDRIAL PROTEIN ALTERATION IN ACTIVE BROWN FAT : A SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORETIC STUDY.

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Received September 29,1976

SUMMARY : The polypeptide composition of mitochondria isolated from the brown adipose tissue of control rats (bred at 22°C) or cold-exposed animals (bred at 6°C) was compared using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A striking increase in the content of an unknown polypeptide (apparent molecular weight 32,000 daltons) was found after cold adaptation, a phenomenon which was reversed during re-adaptation to a normal temperature. This protein seems to be localized in the membrane fraction of the mitochondria.

INTRODUCTION : The existence of a cold-induced increase of the mitochondrial mass of brown adipose tissue is well documented (1-3). This phenomenon presents the double interest to correspond to a peculiar bioenergetic situation since active brown adipose tissue has mainly a calorigenic function, and to offer a possible model for the study of the mitochondriogenesis in cells from higher animals (1-4). We have previously described important cold-induced alterations of the phospholipids and fatty acid phospholipids of the mitochondria from brown adipose tissue (5) and observed, in agreement with others (3, 6), an increase of the mitochondrial protein content ; a concomitant development of the inner mitochondrial membrane has been described, without important increase of the specific oxidative enzyme activities ; the molar composition of the components of the respiratory chain was not modified, except for a slight increase of the concentration in cytochromes and flavoproteins (3, 7). No study of the poplypeptide composition of the mitochondria from this tissue has been reported, this prompted us to perform this analysis. This study

SDS : sodium dodecyl sulfate

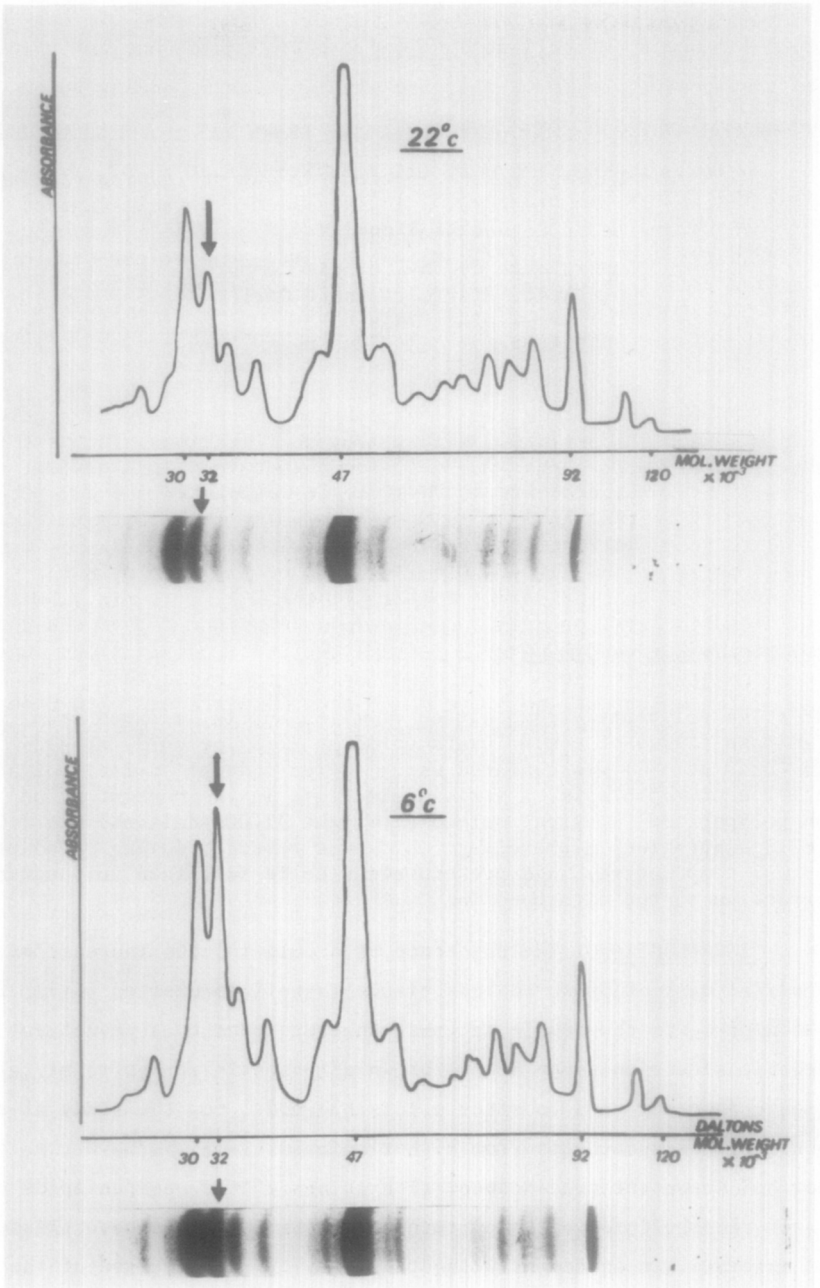

Figure 1 : Comparison of the polypeptide composition of the mitochondria isolated from control rats (22°C) and cold-acclimated animals (6°C).

The densitometric tracings were obtained with 7.5% acrylamide gels stained with Coomassie blue. The molecular weights were calculated according to the electrophoretic mobility of standard proteins (γ - globulin 160,000 - bovine serum albumin 68,000 - ovalbumin 45,000 - chymotrypsinogen 25,000 - myoglobin 17,800 - cytochrome C 12,400). The arrows indicate the position of the cold-increased protein band ; similar results were obtained in studies of 10 different mitochondria pellets for each category of rats. For components of lower molecular weights see the legend of table 1.

was fruitful since a net cold-induced modification which has not been reported previously, was found in a protein band corresponding to an apparent molecular weight of 32,000.

METHODS : Sherman rats bred from the age of weaning (22 days) up to 6 weeks, either at 22°C (control animals) or at 6°C (cold-exposed animals) ; they were fed a normal semi-purified diet. The interscapular brown adipose tissue from 2 or 3 rats was used for the preparation of each mitochondrial pellet which was isolated as previously described (5). The purity of the mitochondrial fraction was found to be good using the determination of glucose-6-phosphatase and acid phosphatase activities and electron microscopy.

SDS-polyacrylamide gel electrophoresis was performed according to the technique of LAEMMLI (8), with slight modifications, in tubes 0.7 cm internal diameter ; the separation gel (7 cm) contained 7.5% or 10% acrylamide and 0.2% bisacrylamide (pH 8.8). The upper concentration gel (0.4 cm) was made from 3% acrylamide and 0.08% bisacrylamide ; SDS 0.1% pH 6.3 was introduced in the gels. The electrode buffer tris-glycine (25 mM - 0.2 M - pH 8.3) contained 1% SDS. After solubilization of the sample in the presence of 2% SDS and 10% mercapteoethanol at 100°C for 3 min., 50 to 100 µg of mitochondrial proteins were introduced at the top of the gel. The electrophoresis was performed at 2 mA/tube until the polypeptides enter the concentration gel, then at 4 mA/tube so long as the bromophenol blue had not reached the end of the gel. The components were stained with Coomassie blue R 250 or Amido black 10B according to FAIRBANKS (9). The bands were scanned at 600 nm (for Amido black) and 540 nm (for Coomassie blue) ; quantitation of the peaks was made by determining their surface areas.

RESULTS AND DISCUSSION :

The mitochondrial protein profile : 17 main polypeptide bands were considered, their apparent molecular weight being distributed between 120,000 and 26,000 daltons (fig. 1, table 1). The exact linearity of the relationship between the logarithm of the molecular weight of standard proteins with a known molecular weight and the electrophoretic mobility relative to bromophenol blue was obtained in the range of 160,000-20,000 daltons for 7.5% acrylamide gels ; below 20,000 it was not strictly linear and subsequently the proteins having a very small apparent molecular weight are not reported in the table, these small polypeptides accounted for a few percent of the total proteins. For the 2 categories, band 11 (47,000 daltons) accounted for 20% of the total proteins, bands 15 and 16 (32,000 and 30,000 daltons) accounted for approximately 10%, the other polypeptides were numerous but less abundant. The first point to consider was the great similarity observed between the protein patterns of control and cold-adapted animals. This observation suggests that in cold-exposure, when the mitochondriogenesis is stimulated the same type of proteins are synthetized (fig. 1, table 1). Only one striking difference could be observed : the protein band number 15 (32,000 daltons) was strongly increased in cold exposed rats comparatively to control animals ; the

TABLE 1 : Polypeptide composition of mitochondria isolated from brown adipose tissue of control rats (22°C) or cold-exposed rats (6°C).

Band number	Mol. weight x 10^{-3}	% total mitochondrial protein	
		22°C	6°C
1	120 ± 4	0.6 ± 0.1	0.5 ± 0.1
2	110 ± 2	1.3 ± 0.1	1.2 ± 0.1
3	92 ± 2	5.2 ± 0.5	4.5 ± 0.3
4	83 ± 2	5.4 ± 0.4	4.9 ± 0.4
5	77 ± 2	3.4 ± 0.3	3.5 ± 0.4
6	71 ± 2	4.3 ± 0.2	4.5 ± 0.3
7	66 ± 1	4.3 ± 0.3	3.8 ± 0.3
8	64 ± 1	3.1 ± 0.2	2.0 ± 0.2
9	58 ± 1	2.7 ± 0.1	2.3 ± 0.2
10	52 ± 1	6.7 ± 0.3	6.1 ± 0.2
11	47 ± 1	20.3 ± 1.1	18.0 ± 1.0
12	44 ± 1	3.9 ± 0.4	4.6 ± 0.4
13	37 ± 1	3.8 ± 0.2	4.5 ± 0.3
14	35 ± 1	5.6 ± 0.3	6.8 ± 0.4
15*	32 ± 1	8.4 ± 0.5	12.4 ± 0.7
16**	30 ± 1	11.8 ± 0.8	10.8 ± 0.6
17	26 ± 0.5	2.0 ± 0.1	2.7 ± 0.2

To each band is assigned an apparent molecular weight (6 determinations, means and s.e.m.) and 2 figures giving its relative importance (% of total mitochondrial protein) in 22°C and 6°C bred animals. These values were obtained with 7.5% acrylamide gel stained with Coomassie blue, very similar results were obtained with 10% acrylamide gels stained with Coomassie blue or Amido black (4-7 determinations). To obtain a better linear resolution of major components around 30,000 daltons (7.5% acrylamide), low molecular weight components comprising cytochrome C were lost or located near by the end of the gel, so it was difficult to identify them ; with 10% acrylamide gels, in conditions where the linearity corresponding to these small compounds was not excellent, the total mass of these small compounds has been estimated to be approximately 7% of the total mitochondrial proteins.

*This difference was highly significant and systematically observed,

**In some cases this band was split into 2 components.

concentration of this protein was increased by 50% (8 to 12%), which is highly significant, and this increase was systematically observed (10 assays) ; the increase of the bands 13 and 14 are not significant. Since cold exposure increases the mitochondrial protein percentage by a factor

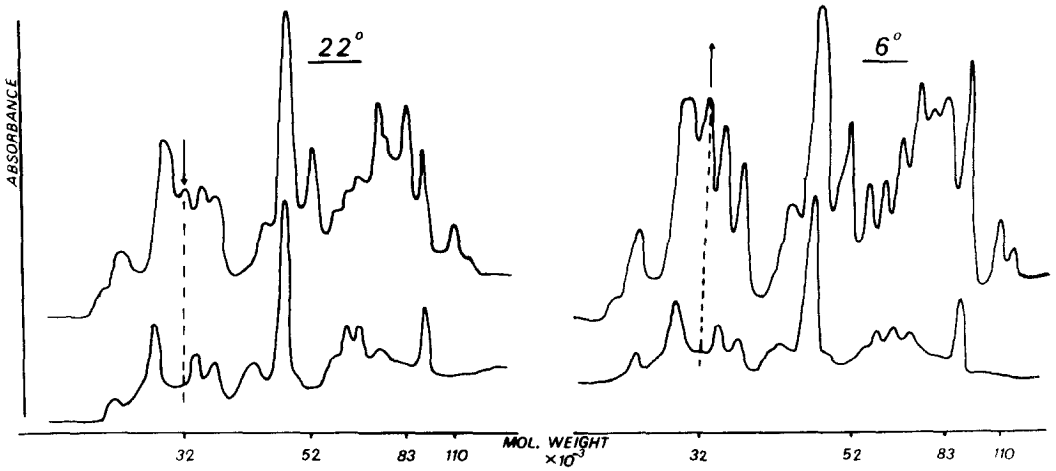

Figure 2 : Effect of KCl treatment on the polypeptide composition of the mitochondria of brown adipose tissue

Control rats (22°C , left part of the figure) and cold-acclimated rats (6°C, right part of the figure). The mitochondria were treated with KCl 0,5M final concentration and left at 0°C for 15 min., the mixture was then centrifuged at 12,000 g per 15 min. The upper tracings correspond to the proteins of the pellet, the lower tracings represent the protein profiles of the supernatants.

1.7 per fresh tissue weight (5), it is possible to calculate that for 100 mg of fresh brown adipose tissue the protein band number 15 can account for 0.35 mg in control rats and 0.88 mg in cold exposed animals.

Effect of cold deadaptation : cold exposed rats have been reexposed at 22°C for 2 weeks and the electrophoretic pattern of their mitochondrial proteins studied in comparison with control and cold exposed animals ; in a small number of experiments the percentages of the protein 15 in control, cold-treated and cold deadapted rats were found respectively to be 9.1-13.5 and 10.2, so the increase by cold of the protein band 15 seemed to have been reversed by cold deadaptation.

Localization of the proteins : the figure 2 shows that after KCl treatment, the protein profile of the pellet (membranes) and of the supernatants (soluble proteins of the matrix and easily extractable membrane proteins) were different. In the supernatants there was less proteins than in the pellet ; moreover it was observed that the protein band 15 (32,000 daltons) was localized in the membrane fraction and firmly bound to it since no protein was found in the supernatants with a position corresponding to band 15.

Since the mitochondrial preparations were clean, it can be assumed that the "polypeptide 15" was associated to mitochondria and not to contaminating fractions. Several problems have not yet been resolved ; is the modification of the polypeptide composition due to the synthesis of a new polypeptide (32,000 daltons) or to the increase of the amount of a pre-existing one ? What is the functional significance of this protein fraction ? Is there any relationship between the increase of this fraction and previous results reporting an increase of some cytochromes and flavoproteins concentrations in these mitochondria after cold exposure (7) ? We have described in a previous paper (5) important alterations of the mitochondrial phospholipids and fatty acids after cold exposure, and in the present work another modification concerning the proteins of the same mitochondria. Is there any correlation between these two findings ? The answer may give informations on the physiological significance of the biochemical changes which do occur when the mitochondriogenesis is stimulated in brown adipose tissue. It is interesting to report that according to HIMMS-HAGEN (10, 11) the biosynthesis of proteins in the mitochondria of brown fat is necessary for the cold-acclimation ; a decreased half-life of some water-insoluble proteins has been also reported in these mitochondria (12). Very recently BUKOWIECKI and HIMMS-HAGEN (13), in a study of the incorporation of L-[U-^{14}C] leucine in the mitochondrial protein of brown fat, have pointed that an altered mitochondrial protein metabolism of brown adipose tissue is associated with the developement of cold-acclimation.

ACKNOWLEDGEMENTS : the skilful technical assistance of Mrs WILLEMIN was gratefully appreciated. We are indebted to Professors P. HEMON and P. MAZLIAK for critical reading of the manuscript and to Miss F. GUERBETTE for the illustrations.

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