IS THERE NUCLEOTIDE CARRIER PRESENT IN OLIGOMYCIN-SENSITIVE ATPase PREPARATIONS?

R. KIEHL

Institut für Physiologische Chemie der Ruhr-Universität Bochum, 4630 Bochum, Universitätsstraße 150, FRG

Received 26 November 1979

1. Introduction

The identity of the uncoupler binding protein (UBP) and the nucleotide carrier have been extensively discussed. Both proteins have mol. wt ~30 000.

The carboxyatractylate (CAT)-binding protein isolated from mitochondria labeled with the tritiated uncoupler 2-azido-4-nitrophenol ([^{3}H]NPA) is reported to yield a product which is completely devoid of radioactivity [1]. Furthermore, coelectrophoresis of mitochondria labeled in the CAT-binding protein with *N*-[^{14}C]ethylmaleide ([^{14}C]NEM) and submitochondrial particles labeled in the UBP with [^{3}H]NPA shows a slight difference in the mobility of the respective isotopes [1].

Here I show that the CAT-binding protein is not present in complex V. Complex V is the segment of the mitochondrial oxidative phosphorylation system which is concerned with ATP synthesis and hydrolysis. In the isolated state, complex V catalyzes oligomycin-sensitive hydrolysis of ATP and oligomycin and uncoupler-sensitive ATP–P_i exchange [2]. The uncoupler sensitivity of complex V suggested that the specific uncoupler binding site of mitochondria might be located in complex V. This was supported by equilibrium binding experiments using the radioactive uncoupler [^{3}H]NPA. There is a capacity of ~0.8 nmol NPA/mg complex V protein [3]. The covalent modification of complex V by [^{3}H]NPA has also been done [4]. As in mitochondria [1] UBP, the α-subunit of F_1, and a small polypeptide are labeled.

N'-(*N''*-*n*-nonyl-4-sulfamoylphenyl)-maleimide (NSPM) has been introduced as lipophilic thiol trapping agent for mitochondria [5,6]. It will be shown that NSPM, beside other functions [7], is able to bind very specifically and covalently to the nucleotide carrier. For this reason it is the reagent of choice to investigate whether the carrier, as well as the uncoupler binding protein, is part of complex V.

2. Materials and methods

Submitochondrial particles (ETP_H) were isolated as in [8]. Complex V [2] and the Serrano type ATPase [9] has been prepared from this ETP_H. Protein was determined by the Lowry method [10]. 12.5% SDS–polyacrylamide gel electrophoresis in the presence of 2-mercaptoethanol was as in [11] and analyzed as in [7].

[^{14}C]NSPM was synthesized as in [6], the specific radioactivity was 3.73 mCi/mmol. Carboxyatractyloside was obtained from Boehringer (Mannheim), diamide from Calbiochem, all other reagents were analytical grade.

3. Results and discussion

Figure 1A shows that [^{14}C]NSPM is able to label 3 different regions in submitochondrial particles:

(i) A protein of mol. wt ~33 000 with a capacity of 0.7–0.8 nmol NSPM/mg: diamide-sensitive. Diamide is able to block the NSPM incorporation into this band by ≤75% (fig.1A, traces 1,2)

(ii) A protein of mol. wt ~30 000 with a capacity of ~2 nmol/NSPM/mg: CAT-sensitive. CAT is able to block the NSPM incorporation into this band by ≤85% (fig.1A, traces 1,3)

(iii) A radioactive peak near the dye front: dialyzable, no protein character (fig.1A, trace 4)

CAT-sensitive thiol labeling is one indication for the

Elsevier/North-Holland Biomedical Press

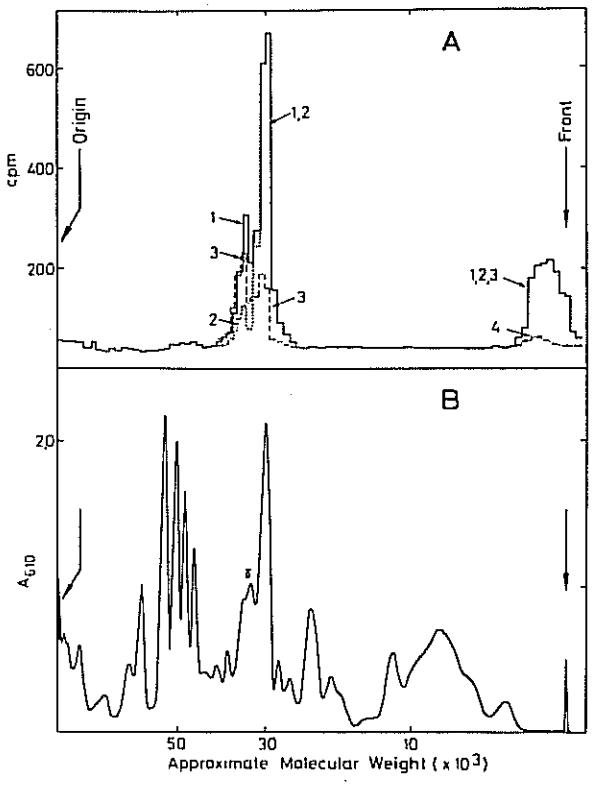

Fig.1. Distribution of radioactivity due to [^{14}C]NSPM in 117 μg submitochondrial particles (A), and distribution of the particle protein (B). Submitochondrial particles were incubated as follows: 0.88 mg particles were added to 1 ml solution containing 0.25 M sucrose, 50 mM Tris–acetate, 30 mM $MgCl_2$, 40 mM phosphate, 12.5 mM ATP (pH 7.5) at 0°C. (1) (solid line) [^{14}C]NSPM was added after 10 min incubation at 0°C and 1 min at 37°C to 20 nmol/mg protein and the reaction stopped after 2 min at 37°C with 1 mM cysteine. (2) (dotted line) as (1) but 10 min preincubation of the particles at 0°C and 1 min at 37°C with 230 nmol diamide/mg protein. (3) (dashed line) as (2) but preincubation with 26 nmol CAT/mg protein. The protein distribution pattern of submitochondrial particles is unchanged by the incubation procedures, the scan shown in fig.1B corresponds to fig.1A, trace 3.

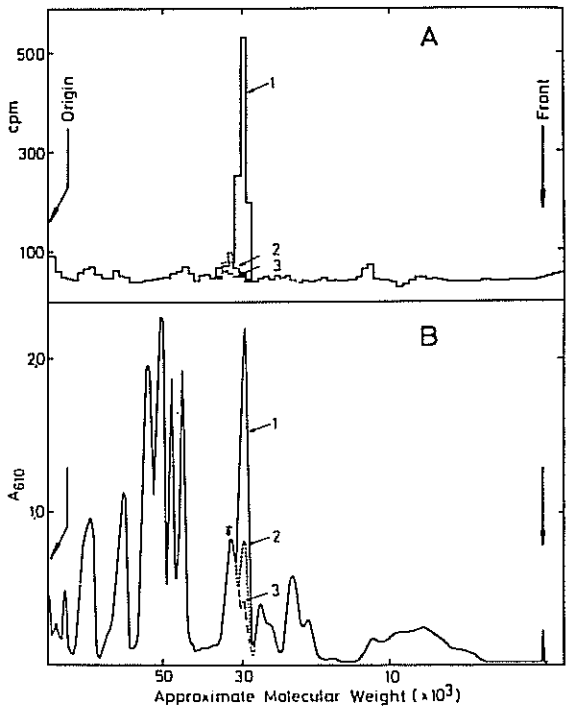

Fig.2. Distribution of radioactivity due to [^{14}C]NSPM in 93 μg ATPase protein (A), and distribution of ATPase protein (B). Conditions as in fig.1. (a) (solid line) 50 nmol [^{14}C]NSPM/mg Serrano-type ATPase protein. (2) (dotted line) as (1) but preincubation of the ATPase with 60 nmol CAT/mg protein. (3) (dashed line) as (1) amount of radioactivity and mol. wt 30 000 band in complex V preparations.

nucleotide carrier in mitochondria [12,13]. It has been estimated on the basis of isolation of the radioiodinated carrier that ~10% (3.4 nmol/mg mitochondrial protein) of the mitochondrial inner membrane proteins consist of the nucleotide carrier. The carrier is therefore the major protein of the mitochondrial inner membrane [14]. The amount of the labeling by [^{14}C]NSPM (2.5–3 nmol/mg protein in mitochondria (unpublished); 2 nmol/mg protein in submitochondrial particles*) compares satisfactorily with this estimate and the number of CAT-binding sites (1.8 nmol/mg mitochondrial protein) [15].

It can be seen in fig.1B that the presence of CAT during incubation results in no apparent difference in the amount of CAT-binding protein in submitochondrial particles. Thus, the inhibition of [^{14}C]NSPM incorporation by CAT is not due to the removal of a specific protein.

Figure 2A shows the labeling pattern of two different kind of oligomycin-sensitive ATPases:

Trace (1): Serrano-type ATPase preparation [9] containing the CAT-binding protein with a capacity of 1.6–1.8 nmol [^{14}C]NSPM/mg protein.

* This value is the highest amount of carrier labeling by [^{14}C]NSPM reached under the present conditions (see figures)

S

Trace (2): CAT prevents completely the incorporation of radioactivity in the mol. wt 30 000 protein of this complex. It may be noted for comparison that the content of DCCD-sensitive proteolipid in this kind of ATPase is ~4–4.5 nmol/mg protein.

Trace (3): Complex V [2] has no NSPM-sensitive CAT-binding protein.

The protein patterns of the Serrano-type-ATPase (fig.2B, traces 1,2) demonstrate that CAT blocks [^{14}C]NSPM incorporation into the complex and effectively removes the carrier from the complex preparation. This shows that the carrier is either loosely attached to the ATPase or only copurified with the ATPase.

The Coomassie blue staining of the mol. wt 30 000 band after CAT treatment is about as high as the staining of the γ-subunit of F_1 (fig.2B/trace 2). Assuming that the staining intensity by Coomassie blue for the γ-subunit and the mol. wt 30 000-band is about the same, it may be concluded that the amount of this protein in the complex is similar to the amount of the γ-subunit.

In complex V preparations the staining intensity of the mol. wt 30 000-band does not exceed the staining of the γ-subunit (fig.2B, trace 3) and no labeling by NSPM has been found in the former band, regardless of the conditions used.

Complex V is an isolated ATP-synthetase preparation catalyzing the highest uncoupler-sensitive ATP–P_i exchange rates reported so far [2]. Since this complex has no CAT-sensitive [^{14}C]NSPM band mol. wt at 30 000 but does contain UBP [4] it can be concluded that the CAT-sensitive nucleotide carrier is not a functional part of the ATP-synthetase complex. This is in contrast to UBP, because the amount of this protein seems to be related to ATP–P_i exchange activity and oligomycin-sensitivity of the complex [4,16,17]. Also, UBP is enriched in F_o-preparations [17,18] and very probably is part of F_o.

In summary the present study shows that (a) the CAT-binding protein is not an integral part of the oligomycin and uncoupler-sensitive ATP–P_i exchange complex, and (b) in contrast, the uncoupler binding protein appears to be an essential part of the oligomycin and uncoupler-sensitive ATP–P_i exchange complex.

Acknowledgements

I thank Dr Y Hatefi, in whose laboratory this work was done and Dr W. G. Hanstein, who helped in preparing the manuscript.

References

[1] Hanstein, W. G. (1979) Methods Enzymol. 56, 653–683.
[2] Stiggall, D. L., Galante, Y. M. and Hatefi, Y. (1978) J. Biol. Chem. 253, 956–964.
[3] Hatefi, Y., Stiggall, D. L., Galante, Y. and Hanstein, W. G. (1974) Biochem. Biophys. Res. Commun. 61, 313–321.
[4] Galante, Y. M., Frigeri, L. and Hatefi, Y. (1978) Front. Biol. Energet. 1, 516–524.
[5] Kiehl, R. and Bäuerlein, E. (1976) FEBS Lett. 72, 24–28.
[6] Kiehl, R. and Bäuerlein, E. (1977) FEBS Lett. 83, 311–315.
[7] Kiehl, R. (1980) in preparation.
[8] Löw, H. and Vallin, L. (1963) Biochim. Biophys. Acta 69, 361–374.
[9] Serrano, R., Kanner, B. I. and Racker, E. (1976) J. Biol. Chem. 251, 2453–2461.
[10] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[11] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
[12] Vignais, P. V. and Vignais, P. M. (1972) FEBS Lett. 26, 27–31.
[13] Aquila, H., Eiermann, W., Babel, W. and Klingenberg, M. (1978) Eur. J. Biochem. 85, 549–560.
[14] Boxer, D. H., Feckl, J. and Klingenberg, M. (1977) FEBS Lett. 73, 43–46.
[15] Riccio, P., Aquila, H. and Klingenberg, M. (1975) FEBS Lett. 56, 133–138.
[16] Berden, J. A. and Voorn, M. M. (1978) Biochim. Biophys. Acta 501, 424.
[17] Alfonzo, A. and Racker, E. (1980) Fed. Proc. FASEB in press.
[18] Galante, Y. and Hatefi, Y. (1980) in preparation.