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FUNCTIONAL RELATIONSHIP BETWEEN THE ADP/ATP-CARRIER AND THE F_1-ATPase IN MITOCHONDRIA

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SUMMARY

1. The distribution of labeled and unlabeled adenine-nucleotides inside and outside mitochondria was followed after addition of [^{14}C]ADP to rat liver mitochondria. Two types of mitochondria were used: 1, respiring mitochondria which were carrying out oxidative phosphorylation and which had been replenished in ATP by incubation in a medium supplemented with succinate and phosphate; 2, non-respiring mitochondria which had been partially depleted of ATP by incubation in a medium supplemented with rotenone and phosphate. During the first minute following addition of [^{14}C]ADP to the respiring mitochondria, the pre-existing intramitochondrial (internal) [^{12}C]ATP was released into the medium and replaced by newly synthesized [^{14}C]ATP. No [^{14}C]ADP accumulated in the mitochondria. It is suggested that extramitochondrial (external) ADP entering respiring mitochondria in exchange for internal ATP is phosphorylated to ATP before its complete release in the matrix space.

In non-respiring mitochondria, the entry of [^{14}C]ADP into the mitochondria was accompanied by the appearance in the external space of [^{12}C]ADP and [^{12}C]-ATP, with a marked predominance of [^{12}C]ADP. Thus in non-respiring mitochondria, the residual internal ATP is dephosphorylated to ADP in the inner membrane before being released outside the mitochondria.

2. When mitochondria were incubated with glutamate, ADP and [^{32}P]phosphate, the [^{32}P]ATP which accumulated in the matrix space became rapidly labeled in both the P_γ and P_β groups of the ATP, due to the presence of a transphosphorylation system in the mitochondrial matrix. The [^{32}P]ATP which accumulated outside the mitochondria was also labeled in the P_β group, although less rapidly than the internal ATP. Our data show that a large fraction (75–80 %) of the ATP produced by phosphorylation of added ADP within the inner mitochondrial membrane is released into the matrix space before being transported out from the mitochondria; only a small part (20–25 %) is released directly outside the mitochondria without penetrating the matrix space.

Abbreviation: EDTA, ethylene-diamine-tetraacetic acid.

3. In respiring and phosphorylating mitochondria, the value of the K_m of the ADP-carrier for external ADP was 2–4 times lower than its value in non-respiring and non-phosphorylating mitochondria.

4. The above experimental data are discussed with reference to the topological and functional relationships between the ADP-carrier and the oxidative phosphorylation complex in the inner mitochondrial membrane. They strongly suggest that the ADP-carrier comes to the close neighbourhood of the ATP synthetase on the matrix side of the inner membrane.

INTRODUCTION

Most of the features of adenine-nucleotide translocation in mitochondria are typical of an exchange-diffusion process in which external ADP and ATP are exchanged for the ADP or ATP of the mitochondrial matrix. However, there are a number of observations which suggest that, under some metabolic conditions, interactions may occur between the ADP-carrier and the coupling system of oxidative phosphorylation which are both located in the inner mitochondrial membrane. They are: (1) The K_m for external ADP [1] and the rate of ADP transport [2] depend on the concentration of internal ATP. (2) The K_m for external ATP and also the competition between ATP and ADP for the ADP/ATP-carrier are dependent on the energy state of mitochondria [3]; (3) The rate of phosphorylation of external ADP by mitochondria is significantly higher than the theoretical rate calculated on the basis of complete equilibration of the added ADP with the pool of internal adenine-nucleotides [4]; (4) Studies performed in the presence of arsenate have been interpreted to indicate that the ATP formed during the oxidative phosphorylation of external ADP is not released into the mitochondrial matrix, but is transported out of the mitochondria [5].

This paper describes the early kinetics of adenine-nucleotide movement across the inner membrane of rat liver mitochondria in different metabolic states. These data provide evidence for a direct interaction between the ADP-carrier and the F_1-ATPase. In addition, studies of the binding of ADP to mitochondria show that the affinity of the ADP-carrier for ADP depends on the state of phosphorylation of mitochondria.

EXPERIMENTAL PROCEDURES

Rat liver mitochondria were isolated in 0.25 M sucrose buffered at pH 7.4 with 1 mM Tris–HCl buffer according to the method of Hogeboom [6].

[^{35}S]Atractyloside, radioactively labeled biosynthetically, was isolated as described previously [7]. [^{14}C]ADP was obtained from Schwarz Bioresearch Inc. (Orangeburg, N.Y., USA), unlabeled nucleotides from P.L. Biochemicals (Milwaukee, Wisc., USA) and phosphoenolpyruvate and the enzymes used for the determination of adenine-nucleotides from Boehringer and Soehne GmbH (Mannheim, Germany). [^{32}P]Phosphate was obtained from the Commissariat à l'Energie Atomique (Saclay, France).

Experiments on adenine-nucleotide translocation were carried out by incubating the mitochondria with [^{14}C]ADP in a medium containing 110 mM KCl,

20 mM Tris–HCl buffer, pH 7.4 and 0.2 mM EDTA (KCl–Tris–EDTA medium). Other additions were made as given in the legends of the figures. After an appropriate time the incubation was stopped by addition of 5 μM carboxyatractyloside, immediately followed by centrifugation at 25 000 $\times g$ for 3 min in a SS-1 Servall centrifuge. This method [8] is derived from the one called atractyloside-centrifugation method [2, 9], where atractyloside instead of carboxyatractyloside was used as an inhibitor to terminate the transport of ADP.

When the phosphorylation pattern of adenine-nucleotides outside and inside mitochondria was to be determined, the incubation was carried out in a medium supplemented with an amount of [^{14}C]ADP in excess to the internal [^{12}C]ADP in order to minimize the changes in specific activity of external [^{14}C]ADP during the course of the incubation; besides, as shown in Results, the level of the internal [^{12}C]ADP did not change very much. EDTA was added to prevent adenylate kinase catalyzed transphosphorylation of the added [^{14}C]ADP. The incubation was ended as follows. An aliquot of the mitochondrial suspension was filtered through a Millipore filter (HAWP, 0.45 μm) fitted to a 5-ml syringe. Filtrate (1 ml) was recovered from each incubation medium within 5 s, and was collected in tubes containing 0.1 ml of 2.5 M perchloric acid. In an exactly parallel experiment, the incubation period was ended directly by addition of perchloric acid to the whole incubation medium. Adenine-nucleotides were assayed in the perchloric extracts after neutralization by KOH [10]. The ATP, ADP and AMP were separated by paper chromatography [11] and their radioactivity determined with a scintillation counter. The difference between the respective amounts of ATP, ADP and AMP found in the whole medium and in the filtrates gave the amount of each nucleotide present within the mitochondrial particles.

When phosphorylation was carried out in the presence of $^{32}P_i$, the time-course of $^{32}P_i$ incorporation into external and internal ATP was followed using the same technique as that described above. The determination of the radioactivity located in the P_β and P_γ of ATP was made after action of hexokinase and glucose [12].

RESULTS

1. *Early stages in the translocation of ^{14}C and ^{12}C-labeled adenine-nucleotides after addition of [^{14}C]ADP to mitochondria*

Respiring and phosphorylating mitochondria. In the following experiments, rat liver mitochondria were preincubated aerobically for 10 min in KCl–Tris–EDTA medium containing succinate and phosphate in order to insure phosphorylation of internal adenine-nucleotides and to raise the level of internal ATP to a steady value. Under these conditions 60–70 % of the total internal adenine-nucleotide was present as ATP. [^{14}C]ADP then was added at a concentration of 70 μM, which considerably exceeds the K_m value for the transport. The distribution of the ^{14}C-labeled adenine-nucleotides derived from the added [^{14}C]ADP and of the non-radioactive adenine-nucleotides derived from the adenine-nucleotides originally present within mitochondria, was measured inside and outside mitochondria, as a function of time.

In a typical experiment as illustrated in Fig. 1, the [^{14}C]ADP added to mitochondria steadily disappeared from the extramitochondrial space. For the first 2 min, the disappearance of external [^{14}C]ADP was accompanied by an accumulation of

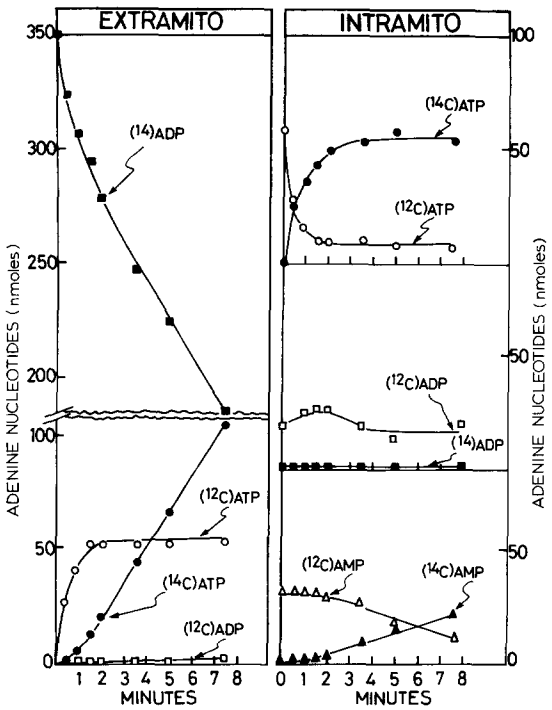

Fig. 1. Movements of ^{14}C- and ^{12}C-labeled adenine-nucleotides across the inner mitochondrial membrane in phosphorylating mitochondria. Rat liver mitochondria (9 mg) were preincubated in 5 ml of 110 mM KCl, 0.2 mM EDTA, 10 mM Tris–HCl, 5 mM phosphate, 5 mM succinate for 10 min at 4 °C. The incubation was started by addition of [^{14}C]ADP to a final concentration of 70 μM Two parallel series of incubations were performed as described in Methods, one being used for the determination of total adenine-nucleotides, the other for the determination of external adenine-nucleotides. The difference between these values correspond to the internal adenine-nucleotides.

[^{14}C]ATP in the intramitochondrial space and by the appearance of [^{12}C]ATP in the extramitochondrial space. The accumulation of [^{12}C]ATP outside the mitochondria stopped after 2 min, as a result of exhaustion of the internal [^{12}C]ATP. Practically no [^{14}C]ADP could be detected in the intramitochondrial space, although there was a constant residual amount of [^{12}C]ADP in this compartment. Two possible explanations can be given to account for these data: (1) The external [^{14}C]ADP is phosphorylated to [^{14}C]ATP before entering the matrix space where it mixes with the pool of internal ATP. (2) The added [^{14}C]ADP enters the mitochondrial matrix, escapes the mixing with internal [^{12}C]ADP, and is phosphorylated so rapidly into [^{14}C]ATP that no [^{14}C]ADP is detected in the matrix. Results obtained with non-respiring mitochondria favor the first alternative (see thereafter).

After a lag of about 1 min, [^{14}C]ATP began to appear in the extramitochondrial space (Fig. 1). After the second min of incubation, the accumulation of [^{14}C]ATP outside the mitochondria proceeded at a steady rate that was nearly equal to the rate of disappearance of external [^{14}C]ADP. In contrast to the changing concentration of internal [^{12}C]ATP, the amount of residual [^{12}C]ADP within the mitochondria remained nearly constant, suggesting a compartmentalization of the

internal [^{12}C]ADP. Although the intramitochondrial level of [^{12}C]ADP remained nearly constant, there was a small, transient, but reproducible, increase during the first min of incubation; this may be relevant to the loss of interaction between F_1-ATPase and the ATPase inhibitor under state 3 condition reported by Van de Stadt et al. [13]. Finally, a slow accumulation of intramitochondrial [^{14}C]AMP was observed after 3–4 min of incubation. This [^{14}C]AMP was most likely derived from the action of a transphosphorylation system present in the matrix space (see below). The presence of EDTA in the incubation medium prevented the action on external adenine-nucleotides of the adenylate kinase which is located in the intermembrane space [14, 15], but was not able to prevent the activity of the transphosphorylation system within the mitochondria since EDTA does not enter the matrix space [16].

A number of experiments similar to that described above were carried out at different concentrations of added [^{14}C]ADP. By using low concentrations of added ADP, it was possible to determine accurately the ratio of ATP to ADP outside and inside the mitochondria after the process of phosphorylation had reached equilibrium. At equilibrium, the ratio of ATP to ADP outside the mitochondria was 7–10 times higher than that inside the mitochondria, in agreement with reports from this and other laboratories [2, 17, 18].

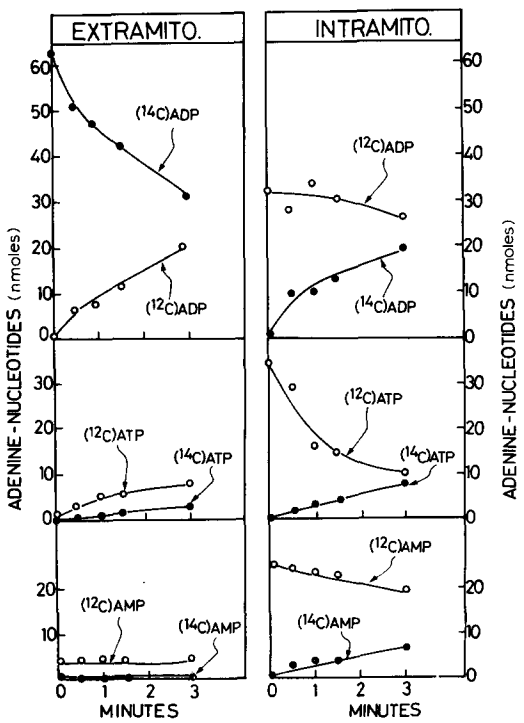

Fig. 2. Movements of ^{14}C- and ^{12}C-labeled adenine-nucleotides across the inner mitochondrial membrane in non-phosphorylating mitochondria. Same experimental conditions as in Fig. 1, except that succinate was replaced by 4 μM rotenone and that [^{14}C]ADP was added to a final concentration of 12 μM.

Non-respiring mitochondria. The same experimental approach as that detailed for the experiment in Fig. 1 has been used to follow the translocation of adenine-nucleotides in mitochondria that have a low respiratory activity as a result of inhibition with rotenone (Fig. 2). Although oxidation of endogenous NAD-linked substrates in these mitochondria was largely prevented by the rotenone, there was some residual oxidation of substrates dependent on flavoprotein-linked dehydrogenases that could account for the fact that ATP constituted about one-third of the total internal adenine-nucleotides at the end of the 10 min preincubation period. However, these non-respiring mitochondria were unable to carry out the oxidative phosphorylation of added ADP.

Most of the added [^{14}C]ADP was transported unchanged into the matrix space (Fig. 2), with only a small fraction being converted into [^{14}C]ATP and [^{14}C]-AMP, probably through the action of the internal transphosphorylation system. The entry of [^{14}C]ADP into mitochondria was accompanied by a marked decrease in the level of internal [^{12}C]ATP, whereas the level of internal [^{12}C]ADP remained unchanged. Concomitant with the decrease in internal [^{12}C]ATP was the appearance outside the mitochondria of [^{12}C]ADP and [^{12}C]ATP with a marked predominance of [^{12}C]ADP. Thus in mitochondria that contained residual ATP, but that were unable to replenish their internal ATP pool by oxidative phosphorylation, the internal ATP was dephosphorylated to ADP in the inner membrane, before being released outside the mitochondria in exchange for external ADP.

The two above experiments, made with respiring and non-respiring mitochondria, respectively, indicate that the ADP-carrier interacts with the F_1 system within the inner mitochondrial membrane; this strongly suggests that F_1 and the ADP-carrier are located adjacent to each other in the membrane.

2. *Kinetics of P_β and P_γ labeling of the ATP present inside and outside mitochondria under conditions of oxidative phosphorylation*

As shown above, external [^{14}C]ADP is phosphorylated to [^{14}C]ATP during its transfer across the inner membrane into the matrix space. This poses the question as to whether the [^{14}C]ATP which later appears outside the mitochondria derives from the same pool of [^{14}C]ATP that had earlier started to accumulate in the intramitochondrial space or whether it derives from phosphorylation of the added [^{14}C]-ADP by some different route which does not involve mixing with the intramitochondrial pool of adenine-nucleotides. This question may be answered by studying the distribution of the ^{32}P-radioactivity in the β and γ phosphate-residues of ATP after incubation of mitochondria with non-radioactive ADP and [^{32}P]phosphate.

Contrary to the experiments with [^{14}C]ADP in the above section, no preincubation was performed in the present experiment, and the incorporation of $^{32}P_i$ into ATP was initiated by adding the mitochondria to KCl–Tris–EDTA medium that had been supplemented with [^{32}P]phosphate and ADP, and with glutamate present as an oxidizable substrate. Two parallel series of incubations were carried out. In the first series, the incubations were terminated by addition of perchloric acid, and in the second series by rapid filtration through a Millipore filter. The amount of $^{32}P_i$ incorporated into ATP referred to both internal and external ATP in the first incubation and to the external ATP in the second one. The amount of $^{32}P_i$ incorporated into the internal ATP was calculated by subtraction. The time course of

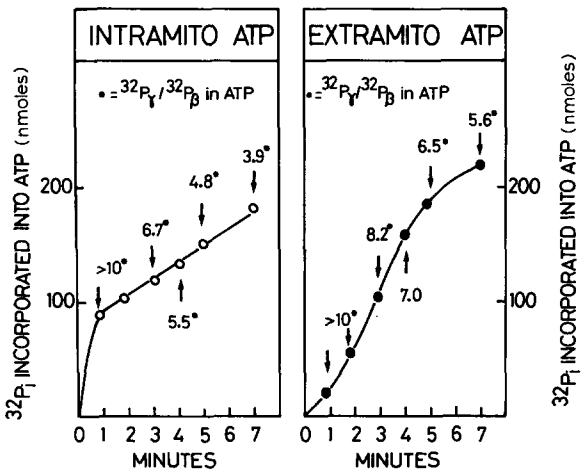

Fig. 3. ^{32}P incorporation into the P_γ and P_β of ATP. (A) External ATP: Rat liver mitochondria (13 mg of protein) were incubated at 4 °C in 110 mM KCl, 0.2 mM EDTA, 10 mM Tris–HCl, 5 mM [^{32}P]phosphate, 40 μM ADP and 10 mM glutamate. The final pH was 7.4 and the total volume 5 ml. Incubation was stopped by rapid filtration through Millipore filter. Filtrates (1 ml) were collected in tubes containing 0.1 ml of 30 % trichloroacetic acid. Numbers with asterisks refer to the ratio $^{32}P_\gamma/^{32}P_\beta$. (B) Internal plus external ATP: Same incubation as above except that incubation was stopped by addition of trichloracetic acid to a final concentration of 3 %. The amount of internal ATP was calculated by subtraction, knowing the amount of external ATP.

incorporation of $^{32}P_i$ into the internal and external ATP is illustrated in Fig. 3, as well as the ratio of radioactivity found in the γ and β phosphate residues. As discussed previously, the labeling of P_β in the internal ATP is most likely due to an endogenous transphosphorylation involving the GTP–AMP phosphotransferase associated with substrate phosphorylation [15, 19] and/or with mitochondrial nucleoside diphosphate kinase [16].

Two possible routes for the formation of external ATP may be considered: 1, that the ATP which is formed by phosphorylation of external ADP within the inner mitochondrial membrane is released directly outside the mitochondria; in this case, the labeling would be located in the terminal phosphate group of the ATP; 2, that the ATP formed from external ADP enters the matrix space and mixes with the doubly labeled ATP derived from internal ADP initially present, before being released outside the mitochondria; in this case the ATP found outside the mitochondria would be labeled in both the β and γ groups. The data in Fig. 3 indicate that, apparently both of these routes are followed with a large predominance of the second one (75–80 %), which involves mixing of the ATP arising from the internal and external ADP. This mixing may explain the small lag in the appearance of [^{32}P]ATP in the extramitochondrial space.

3. *Effect of the phosphorylation state of mitochondria on the K_m^{ADP} for the ADP-carrier*

Souverijn et al. [3] have reported that the K_m of the ADP-carrier for external ATP is strongly dependent on the energy state of the mitochondria, the K_m^{ATP} being more than 100 times greater under high-energy conditions than under low-energy conditions. In contrast, the K_m^{ADP} was found to be much less dependent on the energy state of mitochondria.

TABLE I

EFFECT OF THE STATE OF MITOCHONDRIA ON THE AFFINITY OF THE ADP-CARRIER FOR ADP

Two different media were used. They contained 110 mM KCl, 0.2 mM EDTA and 10 mM Tris–HCl, pH 7.3 and, in addition, (1) for respiring and phosphorylating mitochondria: 5 mM succinate and 5 mM phosphate: (2) for non-respiring mitochondria 5 mM succinate, 5 mM phosphate and 2 μg/ml antimycin A. These media were distributed in tubes (5 ml per tube) and rat liver mitochondria (4 mg per tube) were added and preincubated for 10 min at 6 °C. After preincubation, the K_m of the transport of [^{14}C]ADP was assayed by addition of 100 μl of [^{14}C]ADP at different concentrations. After 15 sec at 6 °C, the incubation was stopped by addition of 6 μM carboxyatractyloside and the suspension was centrifuged immediately. The amount of [^{14}C]ADP incorporated in the matrix space was calculated from the amount of [^{14}C]ADP present in the pellet after correction for the [^{14}C]ADP in the space accessible to sucrose.

State of mitochondria	Internal adenine nucleotide				K_m^{ADP} (μM)
	ATP (%)	ADP (%)	AMP (%)	ATP/ADP	
Respiring and phosphorylating	58	18	24	3.2	0.9
Non-respiring	6	72	22	0.1	4.2

We have compared the affinity for ADP of respiring and non-respiring mitochondria (Table I). The media for the respiring and non-respiring mitochondria contained both succinate and phosphate in order to keep the ionic conditions of incubation similar; the medium for non-respiring mitochondria differed only by the presence of antimycin in a concentration sufficient to inhibit completely the respiratory chain, and consequently to lower markedly the level of internal ATP. In the experiment reported in Table I, the K_m^{ADP} in respiring mitochondria was about 4 times lower than the K_m^{ADP} in non-respiring mitochondria. In each of five similar experiments, the K_m^{ADP} increased from 3 to 5 times during the transition from the respiring state to the non-respiring state. Thus, a state of active oxidative phosphorylation is associated with a higher affinity of the ADP-carrier for ADP.

DISCUSSION

It had been postulated at the early stage of the study on the adenine-nucleotide transport that the transmembrane exchange between external ADP and internal ATP is not connected with the system of oxidative phosphorylation (for review see [20]). This was based on the following observations: (a) internal ADP is phosphorylated sooner than external ADP when anaerobic mitochondria are added to an aerated medium supplemented with ADP [16, 21–23]; (b) the rate of adenine-nucleotide exchange is not significantly modified by uncoupling agents [20]. It was therefore assumed that the oxidative phosphorylation of external ADP takes place within an inner compartment of the mitochondria by the following sequence of reactions: 1 internal ADP is phosphorylated to ATP by the ATP synthetase; 2 external ADP enters the matrix space in exchange for internal ATP and then becomes bound to the ATP synthetase and is phosphorylated into ATP (see Fig. 4B).

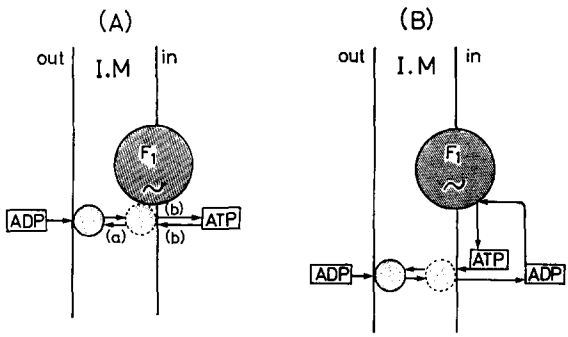

Fig. 4. Interactions between F_1-ATPase and the ADP-carrier. (A) The ADP carrier and F_1 are supposed to be located in the same area of the inner mitochondrial membrane. During its movement back and forth within the inner membrane, the ADP carrier loaded with external ADP comes into close contact with F_1 or moves into a region very close to F_1. The carried ADP effectively reacts immediately with F_1 and is phosphorylated into ATP. This ATP is supposed to follow two routes; either it is taken outside of the mitochondria by the carrier (route a), or it enters the matrix space where it mixes with internal ATP before being carried outside the mitochondria (routes b+a). (B) The ADP carrier and F_1 are supposed to be located in different areas of the inner mitochondrial membrane. The ADP enters the matrix space after transport by the ADP-carrier, mixes with internal ADP before being phosphorylated into ATP by F_1. The newly formed ATP is released into the matrix before being taken up by the carrier to be transported outside mitochondria. Experimental data favor case A.

However the alternative possibility that the ADP carrier interacts with the coupling system of oxidative phosphorylation (see Fig. 4A) has recently received strong support from observations showing that oxidative phosphorylation of external ADP can be accomplished without an obligatory flux of adenine-nucleotides into and out from the mitochondrial matrix [4, 5]. The data presented in this paper suggest that the ADP-carrier, during its movements back and forth, comes into the close proximity and/or close contact with the ATP-synthetase on the matrix side of the inner mitochondrial membrane. Because of this topological relationship the ADP which is carried by the ADP-carrier in respiring and phosphorylating mitochondria is phosphorylated into ATP before being released into the matrix space. This conclusion was arrived from the experiments with added [^{14}C]ADP which showed that external [^{14}C]ADP was phosphorylated to [^{14}C]ATP in the inner membrane before entering the matrix space. Conversely, in non-respiring mitochondria incubated with [^{14}C]ADP, the residual internal ATP is dephosphorylated to ADP by F_1 acting as ATPase during the course of its transport out of the mitochondria. These data taken together strongly suggest that F_1 and the ADP-carrier may interact during the exchange of external ADP for internal ATP and that they are therefore situated in close proximity within the inner membrane (Fig. 4A). When F_1 is functioning as the ATP synthetase, the external ADP is phosphorylated into ATP before entering the matrix space. Conversely, when F_1 is functioning as an ATPase the residual internal ATP is dephosphorylated to ADP before being released outside the mitochondria.

A close interaction between the ADP-carrier and the molecular complex catalyzing oxidative phosphorylation leads us to assume that the two systems behave as a compartmentalized multienzyme system and this may explain the additive in-

hibitory effects of atractyloside and respiratory inhibitors on the 2,4-dinitrophenol-stimulated ATPase and on the ATP–P_i exchange which were described in an early report [24]. It also agrees with the stoichiometry of the amount of ADP-carrier present in mitochondria compared to the amounts of the respiratory components and of the F_1-ATPase. In fact, when titration of the ADP-carrier was carried out with [^{35}S]atractyloside, [^{35}S]carboxyatractyloside, or with [^{14}C]bongkrekic acid, between one and two moles of these inhibitors per mole of cytochrome *a* was found to bind to mitochondria with high affinity [1]. Assuming a one to one stoichiometry for the binding of atractyloside or bongkrekic acid to the ADP-carrier, one may conclude that there is one or at the most two moles of ADP-carrier per mole of cytochrome *a*. A similar value has been reported independently by Bertina and Out [4]. Rat liver mitochondria contain 0.12 nmole of F_1 per mg protein [25], so that the molar ratio of F_1 to cytochrome *a* in rat liver mitochondria is also close to 1. It may therefore be inferred that there is one or at the most two moles of ADP-carrier present per mole of F_1.

Based on the study of the ^{32}P labeling of the P_γ and P_β groups of ATP, it was found that the ATP which is formed within the inner membrane from the added ADP is partitioned between the matrix compartment and the extramitochondrial space. The fact that the greater part of the ATP enters into the matrix rather than being released directly outside the mitochondria suggests that ATP is released as the free nucleotide from the F_1-ATPase into a microaqueous space to which the ADP-carrier also has direct access. This ATP can either diffuse to the matrix or be taken up by the ADP-carrier.

The functional relationship between the F_1-ATPase and the ADP-carrier is emphasized by the fact that the K_m^{ADP} of the ADP-carrier is lower in respiring and phosphorylating mitochondria than in non-respiring mitochondria. The transition from the non-respiring to the respiring state would presumably cause a change of conformation which modifies the affinity of the ADP-carrier for ADP. The fact that the external ADP transported by the ADP-carrier is phosphorylated by F_1 within the inner mitochondrial membrane raises the question of the topological relationship between F_1 and the ADP-carrier. Since evidence has been provided that F_1 and the electron transport chain interact in a direct fashion within the membrane [26], the interaction of the ADP-carrier with F_1 must be considered as extending to the whole oxidative phosphorylation system. Assuming that ADP is transported by a mobile carrier with hydrophobic properties, one may expect that this carrier would move not only across the thickness of the phospholipid bilayer of the inner membrane, but would also move laterally in the plane of the membrane by diffusion between the paraffinic chains of the phospholipids. Since extensive lateral diffusion of the ADP-carrier would prevent the interaction between the oxidative phosphorylation complex and the ADP-carrier from occurring, one is left to assume that such lateral diffusion as takes place must be restricted to the immediate neighbourhood of the oxidative phosphorylation complex. Restriction of lateral diffusion of the ADP-carrier could be due to a specific topological arrangement of components of the oxidative phosphorylation system, so that the ADP-carrier moves back and forth within a volume delimited by the components of the respiratory chain arranged in a loop-like or helix-like assembly in the inner membrane, and then comes into the close proximity of the F_1-ATPase located on the inner side of the inner membrane.

At present our data do not permit us to decide whether the F_1-ATPase interacts directly with the ADP-carrier (a possible interpretation of the experiments with the ^{14}C-labeled nucleotides) or whether there are micro-aqueous spaces to which the F_1-ATPase and the ADP-carrier have direct access and which do not equilibrate rapidly with the matrix space (a concept supported by the experiments with ^{32}P labeling).

The compartmentation of internal ADP has previously been reported [16, 22]. In the experiment illustrated by Fig. 1, the compartmentalized [^{12}C]ADP present in the matrix space represents one fifth of the internal adenine-nucleotides. Compartmentation of ADP by binding to membrane proteins is a possibility and a likely candidate is the mitochondrial ATPase. However, it may be calculated from binding data [27] that less than 5 % of internal ADP and ATP can be compartmentalized by binding to the mitochondrial ATPase.

The transitory rise in internal ADP which occurs at the specific expense of internal ATP following addition of ADP (Fig. 1), and which is possibly due to some hydrolytic activity of F_1, is reminiscent of the dissociation between the ATPase inhibitor and F_1 in respiring submitochondrial particles incubated with ADP [13]. This rise in internal ADP could be accounted for by some of the F_1 molecules in the inner membrane, acting as a hydrolytic ATPase, and hydrolysing selectively some of the ATP contained in the matrix space. One could imagine that, depending on the state of the mitochondria, and on the binding of the ATPase inhibitor to F_1, the catalytic site of F_1 is in contact with the matrix space (ATPase activity) or is oriented more towards the interior of the inner membrane (ATP-synthetase activity).

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REFERENCES

1 Vignais, P. V., Vignais, P. M., Lauquin, G. and Morel, F. (1973) Biochimie 55, 763–778
2 Duée, E. D. and Vignais, P. V. (1969) J. Biol. Chem. 244, 3920–3931
3 Souverijn, J. H. M., Huisman, L. A., Rosing, J. and Kemp, A. (1973) Biochim. Biophys. Acta 305, 185–198
4 Bertina, R. M. and Out, T. A. (1973) Ninth International Congress of Biochemistry, Abstract Book, p. 236
5 Bertagnolli, B. L. and Hanson, J. B. (1973) Plant Physiol. 52, 431–435
6 Hogeboom, G. (1955) in Methods in Enzymology (Colowick, S. P. and Kaplan, N. O., eds), Vol. I, pp. 16–19, Academic Press, New York
7 Vignais, P. V., Vignais, P. M. and Colomb, M. G. (1970) FEBS Lett. 8, 328–332
8 Vignais, P. V., Vignais, P. M. and Defaye, G. (1973) Biochemistry 12, 1508–1519
9 Vignais, P. V. and Duée, E. D. (1966) Bull. Soc. Chim. Biol. 48, 1169–1187
10 Adam, H. (1963) in Methods in Enzymatic Analysis (Bergmeyer, H. V., ed.), pp. 539–573, Academic Press, New York
11 Duée, E. D. (1968) Bull. Soc. Chim. Biol. 50, 1215–1219
12 Vignais, P. V., Duée, E. D., Vignais, P. M. and Huet, J. (1966) Biochim. Biophys. Acta, 118, 465–483

13 Van de Stadt, R. J., De Boer, B. L. and Van Dam, K. (1973) Biochim. Biophys. Acta 292, 338–349
14 Sottocasa, G. L., Kuylenstierna, B., Ernster, L. and Bergstrand, A. (1967) in Methods in Enzymology (Estabrook, R. W. and Pullman, M., eds), Vol. X, pp. 448–463, Academic Press, New York
15 Silva-Lima, M. and Vignais, P. V. (1968) Bull. Soc. Chim. Biol. 50, 1833–1848
16 Duée, E. D. and Vignais, P. V. (1969) J. Biol. Chem. 244, 3932–3940
17 Heldt, H. W., Klingenberg, M. and Milovancev, H. (1972) Eur. J. Biochem. 30, 434–440
18 Slater, E. C., Rosing, J. J. and Mol. A. (1973) Biochim. Biophys. Acta, 292, 534–553
19 Tokumitsu, Y. and Ui, M. (1973) Biochim. Biophys. Acta 292, 310–324
20 Klingenberg, M., Wulf, R., Heldt, H. W. and Pfaff, E. (1969) in Mitochondria Structure and Function (Ernster, L. and Drahota, Z., eds), Vol. 17, pp. 59–77, Academic Press, New York
21 Heldt, H. W., Jacobs, H. and Klingenberg, M. (1965) Biochem. Biophys. Res. Commun. 18, 174–179
22 Vignais, P. V. and Duée, E. D. (1966) Bull. Soc. Chim. Biol. 46, 1169–1187
23 Heldt, H. W. (1966) in Regulation of Metabolic Processes in Mitochondria, B.B.A. Library 7, pp. 50–64, Elsevier, Amsterdam
24 Vignais, P. V., Vignais, P. M. and Stanislas, E. (1962) Biochim. Biophys. Acta, 60, 284–300
25 Bertina, R. M., Schrier, P. I. and Slater, E. C. (1973) Biochim. Biophys. Acta 305, 503–518
26 Ernster, L., Juntii, K. and Asami, K. (1973) Bioenergetics 4, 149–159
27 Harris, D. A., Rosing, J., Van de Stadt, R. J. and Slater, E. C. (1973) Biochim. Biophys. Acta 314, 149–153