REVIEW LETTER

MITOCHONDRIA METABOLITE TRANSPORT

M.KLINGENBERG
Lehrstuhl für Physikalische Biochemie Universität München, Goethestrasse 33, 8 München 15, Germany

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1. Introduction

It is only during the last 4 to 6 years that the functional consequences of mitochondria being a largely closed membrane-surrounded system have been fully recognized. The present report intends to review these developments with particular attention to the metabolite transport systems. The occurrence of the great variety and activity of these transport systems can be attributed to the close interlinkage of mitochondria with the hyaloplasma. Research on these carrier systems is in full progress and our knowledge of most of them is still fragmentary. This brief review can only give a preliminary account of mitochondrial metabolite transport systems.

2. Methods for studying metabolite transport in mitochondria

Indirect methods for following metabolite transport have made use of reactions in mitochondria which appear to be limited by the transport reaction. Substrate-induced changes in the redox state of the intramitochondrial NAD and NADP systems were used early on as indicators of the penetration of these substrates and even for ADP and P_i [1–4]. The results are of limited value since it often remains doubtful whether reactions other than transport are rate limiting. A method indicating substrate uptake more directly is based on the light scattering changes of mitochondrial suspensions which can be attributed to an osmotically driven swelling of the mitochondria [5]. This method is limited to the net uptake of metabolites and therefore does not permit measurement of exchange unless coupling to a net transport is possible, as, for example, in malate uptake coupled to a phosphate-OH^- shuttle. The method is mostly used for concentrations much higher than physiological and therefore these results must be extrapolated with caution. Only semiquantitative and relative values for permeation rates can be obtained. The advantages of the method are the extreme ease of application, only a recording photometer being required.

The direct methods require in general a separation of the mitochondria from the incubating medium for assaying the distribution of the permeant substrate. The techniques are more complex, particularly if they require short penetration times for kinetic analysis and instantaneous quenching of the mitochondrial enzyme activity to prevent further interconversion of the permeated metabolite. Various types of separation methods have been developed, in particular in studies on the adenine nucleotide translocation [cf. 6]. The centrifugation filtration method in which mitochondria are centrifuged through a silicone layer into a quenching bottom layer of acid [7, 8] has been particulary useful. A shorter time of separation has been achieved with multiple layers [9]. The centrifugal filtration method can also be used for measuring "back" or "steady state" exchanges. The simultaneous quenching and separation permits measurement of the internal "pattern" of the metabolites, such as the phosphorylation pattern of adenine nucleotides [6, 10]. In this way the specificity of the back reaction in the adenine nucleotide exchange could be measured [6].

Particularly useful for kinetic measurements is a method in which the transport is stopped by addition of a transport inhibitor [6]. Subsequently the mitochondria are separated by centrifugation for measure-

ment of substrate distribution. The metabolite pattern, however, is not retained in this method. In combination with rapid mixing devices, continuous sampling [11], a resolution down to 1 sec for the penetration can be achieved.

3. Permeation properties of the inner and outer membrane

In early research on permeation into mitochondria, sucrose and chloride, often the main constituents of the incubation medium, were found to penetrate a large part of the mitochondrial space, excluding a "sucrose-impermeable space" [7, 12]. The impermeable space increased on lowering the external sucrose concentration, in contrast with the permeable space and thus behaves as an osmotically active space [13, 14]. Correlation with morphological changes of the mitochondria demonstrated [15] that the matrix space corresponds to the osmotically active space. Consequently the inner mitochondrial membrane was shown to be impermeable to sucrose as well as to many other hydrophilic solutes [10, 15]. The permeable space can thus be identified with the intramembrane space and its accessibility attributed to the unspecific permeability of the outer membrane, which appears to be a barries only to molecules with MW < 5000 [15]. It remains an open question whether the permeability of the outer membrane reflects the situation *in vivo* or is an artefact of isolation of the mitochondria due to the high fragility of this membrane. It apparently encloses a number of soluble enzymes localized in the intramembrane space, in particular phosphate transferases, such as adenylate kinase, creatine kinase, nucleoside diphosphate kinase [10]. This has been confirmed by the digitonin procedure for dissolving the outer membrane [16].

4. The inner membrane as a selective barrier

The general impermeability of the inner membrane to most metabolites is reflected by the mere fact that mitochondria can be isolated which apparently retain their full complement of nucleotides, coenzymes, as well as some ions and metabolites. A number of these metabolites are exchanged through the inner membrane by specific transport systems (cf. table 1). From these specific systems, the unspecific permeability of the inner membrane, which is similar to those of other "tight" biological membranes, such as erythrocytes, must be differentiated. The inner membrane is permeable to water, monovalent acids such as pyruvate, hydroxybutyrate, acetoacetate, acetate, fatty acid, and to some polarizable anions such as azide [17] and SCN^- [18]. Permeation of these substances has been followed by swelling methods or by their effects on the intramitochondrial components. A number of results indicate that most of the monovalent acids or anions may permeate in the undissociated form [5, 19]. Although the undissociated fraction for the monovalent acids at pH 7 is in the order of 10^{-2} to 10^{-3}, it is still much lower for the dicarboxylic acids (10^{-5} to 10^{-7}) [19].

The unspecific permeation of acetate does not appear to be "saturated" in a concentration range up to 50 mM, in contrast to that of carrier-dependent substrates [20]. The rate of permeation as judged from relative measurements of osmotic swelling, is considerably higher than that for carrier-driven substrates [20]. The transport of acetate has been coupled to the uptake of cations such as K^+, Ca^{2+}, as a charge-compensating anion with a penetration rate which does not limit the cation uptake [21, 22]. It appears that other monobasic metabolites of mitochondria, such as pyruvate, acetoacetate and hydroxybutyrate, are also transported without carrier, however, with less activity than acetate. The evidence is based in part on the absence of a specific inhibitor for the uptake of puryvate. It appears that at high pH (> 8) the inner membrane also becomes unspecifically permeable to Cl^- and succinate [23]. Apparently in these conditions there are aqueous pores in the membrane.

5. Exogenous impermeable metabolites

Some substrates are oxidized by mitochondria without penetrating the matrix. In mitochondria from yeast, external NADH is actively oxidized without entering the matrix and without exchanging with the endogenous NAD [24, 25]. The existence of an externally directed NADH-dehydrogenase could be demonstrated. Similarly glycerol-1-phosphate does not permeate mitochondria from various sources, al-

Table 1
Survey of mitochondrial permeant metabolites.

Metabolites	Permeant	Inhibitors	Carrier	Remarks
Oxidative phosphorylation	ADP	Atractyloside	+	Exchange
	ATP	Bongkrekic acid	+	
	P_i	SH-reagent	+	
Substrates	Pyruvate	–	–	(Indirect transport by carnitine shuttle)
	Fatty acids	–	–	
Ketone bodies	Hydroxybutyrate	–	–	
	Acetoacetate	–	–	
Intermediates				
Dicarboxylates	Malate	Butylmalonate	+	Exchange with P_i
	Succinate		+	
	Ketoglutarate		+	
Tricarboxylates	Citrate	–	+	Exchange with dicarboxylates
	Isocitrate	–	+	
Amino acids	Aspartate	Avenoceolide	+	Activator: glutamate
	Glutamate		+	

though it is oxidized by a dehydrogenase which is located at the external surface of the inner membrane [25, 26].

6. Specific metabolite transport through the inner membrane

The transport of impermeable metabolites through the inner membrane is catalyzed by carrier systems which have also been called translocases, permeases, porters, etc. The major carriers belong to the metabolite exchange between mitochondria and hyaloplasma, associated with oxidative phosphorylation and the tricarboxylic acid cycle. There are further carriers for the uptake of amino acids, etc. Criteria to be met for the carrier systems are specificity, saturability, the existence of specific inhibitors, high temperature dependence. These criteria are fulfilled by the adenine nucleotide translocase and, in part, by other transport systems.

The carriers linked to oxidative phosphorylation have been found in all mitochondria tested so far. In contrast, carriers of the tricarboxylic acid intermediates and of amino acids are found in mitochondria from more specialized organs, in particular the liver. The organ dependence of the occurrence of some carriers, such as the dicarboxylate carrier, supports the suggestion that the observed substrate uptake reflects some specific and physiologically important mechanism and is not only an *in vitro* effect of the mitochondrial membrane.

The selective permeation of phosphate and some dicarboxylic acids such as malate, malonate and succinate, was postulated early on in context with the oxidation of intramitochondrial NADPH and NADH by reductive amination of ketoglutarate [2, 27–29]. It has been confirmed that these selective permeations are caused by anion transport of dicarboxylic acid in exchange for ketoglutarate [30, 31]. The same procedure was applied later in studies on the permeation of glutamate, ketoglutarate and aspartate [32, 33]. The distribution of aspartate, malate and ketoglutarate was measured early on by the centrifugal filtration method and found to be unequally distributed in the intra- and extramitochondrial space, in particular under the influence of energy [1]. Different redox potentials of the intra- and extramitochondrial redox systems of malate/oxaloacetate, glutamate/ketoglutarate + NH_3 were measured, in particular under the influence of ATP.

The uptake of succinate has been measured in as-

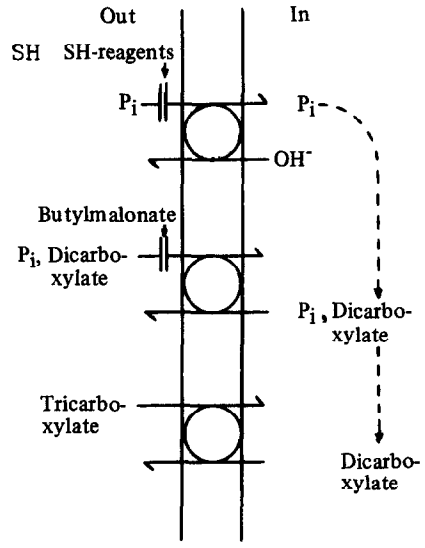
sociation with movement of Ca^{2+} and K^+ [21, 22, 34]. Furthermore, the rate of succinate oxidation could be quantitatively correlated to succinate uptake which controls the internal succinate concentration [35]. Direct studies on the uptake of succinate with the centrifugal filtration method gave a more detailed picture of the uptake of various anions under stationary conditions, in particular demonstrating competition with various anions.

The stationary distribution of inner metabolite compared to external metabolites has been studied under various conditions and viewpoints [14, 35–37]. Thus various anions were found to compete in the mitochondria for the capacity of mitochondrial loading. The "affinity" increased form the mono- to the tricarboxylates [14, 35, 38]. This competition could be directly correlated to a control of the metabolic utilization of the permeant ion in the case of succinate oxidation [35].

An insight into the active carrier system was gained by radically simplifying permeation studies by applying relatively crude methods of monitoring osmotic swelling due to massive uptake of metabolites (cf. 19, 39, 40]. One version of this technique is to follow the swelling of the mitochondria induced by the uptake of K^+ in the presence of valinomycin, which requires the presence of a permeable anion and respiration [20, 21, 22]. The advantage of the method is the use of relatively low external anion concentrations. However, the respiratory substrate must also be taken up at a sufficiently high rate and may compete with other anions. With high anion concentrations, near the iso-osmolar range, osmotic swelling can be measured independently of the energy supply [5, 19, 39]. In this case the osmotic swelling reflects the equilibration of the externally added solutes with the internal space in the presence of permeable NH_4^+ or K^+ plus valinomycin [18]. Ammonium salts have been widely applied in the qualitative screening of permeation of anions.

By this method three transport carriers for the permeation of tricarboxylic acid cycle intermediates have been distinguished based on substrate specificity, activators and inhibitors of the translocation [cf. 19, 41]. The dicarboxylic carrier has a relative specificity, the most active being L-malate and malonate, succinate, D-malate, but not for other dicarboxylates such as fumarate, maleate. The carrier system for tricarboxylates is specific for citrate, isocitrate, cis-aconitate. These carriers require specific activators for maximal activity: the dicarboxylate carrier needs P_i, the tricarboxylate carrier needs a permeant dicarboxylate plus P_i. Thus there is a sequential interlinkage between the carrier for phosphate, dicarboxylates and tricarboxylates, probably by reciprocal exchange reactions, as shown in scheme 1. Mainly on the basis of the activator requirements another carrier for ketoglutarate dif-

Scheme 1



ferent from the dicarboxylate and tricarboxylate carriers was defined [30]. Common activators for both ketoglutarate and tricarboxylic carriers are malate-succinate whereas only malonate activates ketoglutarate entry and only iso-malate activatives the entry of citrate [41]. The nature of this activation could be elucidated by applying the procedures developed for the adenine nucleotide (AdN) translocation to the substrate transport. A 1 : 1 exchange between the added and the intramitochondrial anions [42, 31], in analogy to the AdN exchange, was first demonstrated by Palmieri [42] for citrate-malate exchange. The method was also used [31] for demonstrating the malate-ketoglutarate [31] and malate-P_i [43] exchange. Also early results by Gamble [44] indicate that there is an exchange between citrate, malate and P_i.

The existence of exchange carriers can be rationalized in terms of the osmotic equilibrium of the matrix space. Only the net uptake of P_i is not coupled to an exchange. The cascade-like coupling of the di- and

tricarboxylate exchange carrier to the P_i carrier would also permit a net uptake of these substrates by a cycling of P_i. If substrate transport is a strictly coupled exchange, any net uptake should be caused only by the P_i-shuttle. P_i may originate, if not added, from endogenous mitochondrial P_i.

Important support for the carrier concept of transport of these metabolites was obtained by identifying inhibitors. Butylmalonate and phenyl-succinate were found to inhibit the dicarboxylic carriers [45]. Furthermore, the inhibition of oxidative phosphorylation by SH-reagents in intact mitochondria was primarily due to inhibition of the phosphate carrier by SH-reagents [46–48]. Contradictory data were available on the effects of SH-reagents and the interlinkage between the dicarboxylate and phosphate transport. Butylmalonate was reported [49] to inhibit the dicarboxylate-phosphate exchange but not the dicarboxylate-dicarboxylate exchange and SH-reagents were found to inhibit both the single phosphate carrier and the dicarboxylate-phosphate carrier [49]. Chappell [50] concluded, however, that the phosphate-phosphate exchange is insensitive to SH-reagents, but sensitive to butylmalonate.

The two most important amino acids linked to mitochondrial metabolism, glutamate and aspartate, are also transported by specific carriers: glutamate is transported by a carrier apparently without an activating exchange ion [32]. Of great interest is the finding of a highly specific inhibitor for this carrier, the antibiotic avenoceolide [51]. Aspartate cannot penetrate the mitochondria except in the presence of glutamate [33]. This is in agreement with direct studies on the non-permeability of aspartate [15, 33].

Only recently [52, 53] attempts have been made to measure the kinetics of the dicarboxylate uptake in mitochondria according to the procedures established for the adenine nucleotide translocation. Two methods were used: a modified layer filtration method, in which mitochondria are injected into an incubation layer and then filtred through silicone [52], and the inhibitor-stop method using phenyl-succinate to inhibit succinate uptake [53]. The latter method has the advantage that a minimum of 1 sec can be resolved with simple centrifugation techniques. From these methods, the rate of succinate uptake in liver mitochondria is obtained: for succinate (10°C V_{max} = 50 μmoles/g/min, K = 0.8 mM [53], and for malate (0°C) K_m = 1.4 mM. Further kinetic studies of evaluating the elementary kinetic parameters are urgently needed in order that a more quantitative and unequivocal description is attained and the still phenomenological stage in this part of the metabolite transport is overcome.

7. Factors determining anion distribution

It is accepted that the anion carriers are not comparable to energy-driven carriers for example, the ATP-driven uptake of glucose in *E. coli*. However, an active transport can be catalyzed by these carriers when a distribution of the metabolites is established following an energy-linked gradient of cations or pH difference. It has been visualized that the anions follow the actively transported cations compensating for the cationic charges [54, 55, 37]. In other words, the membrane potential created by the energy-driven potassium pump which is positive inside would attract the anions. From the anion distribution, according to this mechanism, an inside positive membrane potential of about 25–40 mV has been calculated [56]. However, a number of results argue against this interpretation. The anions can often be taken up by a charge compensating exchange and, in particular in the case of phosphate, the uptake can be neutralized by H^+ or exchange for OH^-. In studies comparing the effect of ionophores such as nonactin, nigericin, on the pH difference in the mitochondria, the distribution of succinate and malate was shown to follow the pH gradient and not the K^+ gradient across the membrane [35]. The relation between the anion distribution for mono-, di- and tricarboxylic acids was found to fit the assumption that the pH difference causes the anion accumulation, within the limit of the mitochondrial capacity for anion uptake [57] [cf. also 18]. The cation uptake driven anion movements are then explained by the pH differences created by the cation movements. At very low anion concentration, in the ideal case, the following relation should hold:

$$\frac{A_i^{n-}}{A_o^{n-}} = \frac{(H^+)_o^n}{(H^+)_i^n}$$

This equation can be arrived at from the concept that

anions move through the membrane in the unionized form rather than as anions [see also 58, 59]. It may be conceived that the H^+ is not attached to the carboxylate but rather to a basic group on the carrier. The net effect on the anion distribution would be the same.

8. Adenine nucleotide translocation

Adenine nucleotide (AdN) translocation was the first metabolite transport in mitochondria to be defined as a carrier-driven process [10, 60]. Research on this process has pioneered the development of new methods for measuring metabolite translocation, setting the stage for a quantitative evaluation of the mitochondrial metabolite transport. The kinetics of this system have been investigated and data on kinetic parameters, on the regulation and on the identification of the carrier sites obtained [6, 11]. Furthermore, it was established early on in context with the AdN translocation, that the inner membrane is the site of metabolite carrier systems [10, 61].

The unspecific permeation into the intramembrane space has been distinguished from the specific translocation by the time and concentration dependence [10, 62]. Unspecific permeation is complete at low temperatures within a few seconds, and increases linearly with outside concentration, whereas the AdN exchange is incomplete even after several minutes and is already saturated at $< 50\ \mu M$.

A particular factor in AdN translocation is the large endogenous pool already present in the mitochondria [63–65]. Exogenous AdN is transported in a 1 to 1 exchange ratio with the endogenous AdN, so that the size of the endogenous AdN pool (ATP + ADP + AMP) remains constant for short periods. AdN translocation excludes transport of AMP both from ouside [19] as well as from inside [6]. The system is highly specific for ADP and ATP with some allowance for the deoxyribose nucleotide. Thus nucleotides with bases other than adenine are not translocated. The high specificity and affinity of the mitochondria for ADP is superimposed by translocation on a rather unspecific phosphorylating enzyme in the mitochondria.

ATP was shown early on by double labelling to be exchanged as an intact molecule [6, 10], a finding which disproves the transphosphorylating "mesomerases" theory, proposed by Brierley and Green [66], and Chappell and Crofts [3], and which is still found in textbooks. The specific AdN exchange has also been found in a number of mitochondria from other sources [67, 68]. The exchange is much faster, particularly in mitochondria from heart [67], so that no reliable rate data are available yet.

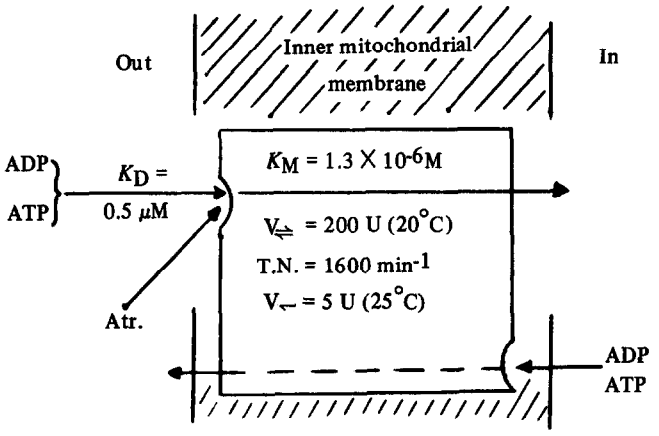
A useful tool has been atractyloside, which was shown to be a specific inhibitor of the translocation [10, 60]. The finding of Bruni et al. [69] that atractyloside inhibits the binding of AdN by the mitochondria could thus be interpreted in the light of the AdN translocation. In subsequent studies, Duée and Vignais [70] as well as Winkler et al. [71] also demonstrated an inhibition of the "binding" by atractyloside. A number of atractyloside analogues were found also to inhibit the AdN exchange, however, with less efficiency [72]. Chappell and Crofts [3] also found that atracyloside interferes with the utilization of exogenous AdN by the mitochondria. Without measuring the actual translocation they were unable to define this process an an exchange. Since they did not differentiate between the permeability of the inner and outer membrane, they proposed that the AdN permease was located between the inner and the outer membrane. A related conclusion comes from binding studies with atractyloside by Vignais [73] (see below). However, the main body of evidence remains in favour of an localization of the atractyloside-sensitive translocation at the inner membrane.

The use of excessively high concentrations of atractyloside by Chappell and Crofts [3] resulted in some confusion about the compartmentation of fatty acid activation [74, 75]. It has now been shown by Alexandre et al. [76] that atractyloside in high concentrations also inhibits fatty acid activation enzymes. Therefore the conclusion about multiple compartmentation [74] of fatty acid activation based on an "atractyloside barrier" seems to be superseded.

The quantitative understanding of the AdN exchange was promoted by the finding that the translocation from the inside also excludes AMP, whereas the endogenous ADP and ATP are equally active [6, 10]. Thus the total endogenous pool is functionally compartmented into AMP and ADP + ATP portions which vary under different metabolic conditions. Based on the size of the ADP + ATP pool, a quantative evaluation as a first order reaction has been possible and true translocation rates (in μmoles/min) have

been obtained independent of the variable (ADP + ATP) pool [11]. Various kinetic parameters evaluated on this basis are given in Scheme 2. The high specificity, the very low K_m and the very high temperature

Scheme 2
Summary of data on the AdN carrier



Further data: *Exchange rate* (ADP) V = 7 U (0°C), 200 U (20°C) (U = μmoles/g prot/min). *Control ratio* ($V_{controlled}/V_{uncoupled}$ state: for exog. ATP = 0.1 to 0.3; for exog. ADP, endog. ADP, ATP = 1. *Activation energy* E_a (kcal) = 21 (0–8° C), 34 (8–20°C). *Carrier sites*: 0.15 μmoles/g prot. 1 site/cyt. *a*. K_d (ATP) = K_d (ADP) = 0.5 μM. *T.N.* (min^{-1}) = 50 (0°C). extrapolated 2600 (25°C), 9500 (37°C).

dependence of AdN translocation are unusual factors for AdN specific enzymes.

Oscillations in the rate of the AdN translocation have been claimed by Duée and Vignais [77] but are not convincingly demonstrated by their actual data. Their results on specificity, AdN pool size, temperature dependence, largely agree with the results of Pfaff Klingenberg, Heldt [6, 10, 68]. In contrast, Winkler et al. [71] find a much higher rate of AdN binding, probably due to experimental problems in their filtration methods. The "specific" leakage of AdN through mitochondria has been attributed to a one-sided in → out function of the carriers, under conditions where only AdN and no other nucleotides, coenzymes, etc. leak from the mitochondria [78]. This leakage also excludes AMP and is inhibited in the uncoupled state. The ratio of single to exchange rate obtained is 1 : 100. Vignais [79], however, arrived at the conclusion that leakage is a process not catalyzed by the carrier system.

Of particular interest is the observation that added ATP is exchanged considerably slower than ADP, unless the mitochondria are uncoupled [6, 10]. In particular under competitive conditions, ADP exchanges about 10 to 15 times faster than ATP [6, 80]. It was concluded that this discrimination between ADP and ATP is superimposed by an energized state of the membrane upon the basically equal specificity of the carrier for ADP and ATP [81, 82]. In contrast, from the inside the specificity for both is equal under all conditions. It was suggested that a charge discrepancy in the ATP^{4-} against ADP^{3-} exchange leads to a net unneutralized influx of one negative charge [83]. Thus, in the energized state, a membrane potential negative inside would inhibit this exchange.

9. Extramitochondrial ATP and ADP under the control of the AdN exchange

The great preference of ADP as compared to ATP enables the mitochondria to phosphorylate ADP efficiently, even in a high excess of ATP. This leads to an asymmetric distribution of the nucleotides with a higher ATP/ADP outside than inside. Consequently the phosphorylation potential of the ATP system is 2 to 4 kcal higher outside than inside. This should lead to a decrease of the P/O ratio, so that less ATP is produced but at a higher potential, thus conserving the total free energy [82]. It has been shown by double labelling experiments [84, 85, 86] that the phosphorylation of the endogenous AdN precedes that of the exogenous portion and therefore the exchange is on the main pathway of the phosphorylation of exogenous AdN. With the new values for the rate of translocation, the rate of phosphorylation of exogenous AdN has been quantitatively analyzed as a function of the translocation rate and the phosphorylation and dephosphorylation of endogenous AdN [87].

The barrier set for AMP requires that separate adenylate kinase systems exist inside and outside the inner membrane. The adenylate kinase of liver mitochondria has been located in the intramembrane space together with other phosphate transferases [10, 16]. Therefore intramitochondrial AMP generated by substrate activation would be trapped. In fact, for the

conversion of AMP to ADP, a nucleoside monophosphate kinase system linked to substrate level phosphorylation has been shown to exist in mitochondria [88].

10. Molecular definition of the carrier

The extremely high affinity of translocation [6] for ADP suggests that the carrier site might be defined by directly measuring the binding. In order to discriminate sufficiently from the excess of endogenous AdN, the mitochondria must be largely depleted. With the help of atractyloside, specific binding sites can be elucidated and linear binding curves obtained for evaluation of the number of sites and the K_m [89]. In previous studies by Winkler et al. [90] on detergent treated membranes, exchange and binding were not convincingly distinguished. Therefore, these authors concluded that atractyloside and AdN attach to different sites and that atractyloside exerts its inhibition by an "allosteric" effect.

An additional equal number of sites with a lower affinity (K_m = 1.5 μM) was found in beef heart mitochondria, possibly identical with the inner sides of the carrier [91]. The affinity is equal for ADP and ATP. Studies with atractyloside indicate an about equal number of atractyloside and of AdN bound [91, cf. also 92]. In contrast, Vignais [73] finds most of the atractyloside binds to the outer membrane and postulates that atractyloside inhibition is caused by an allosteric effect from the outer binding site on a multitude of inner membrane AdN carrier sites, in extension of earlier studies with atractyloside analogues [72].

11. Outlook

The research on mitochondrial metabolite transport will continue on several lines. The molecular definition of a carrier is at present the main problem of research on biological membranes. Mitochondria with their high activity and diversity of carrier catalyzing membrane transport appear to be particularly suited for the definition of a carrier, the elucidation of its mechanism and its eventual isolation. It is the opninion of this review that the largest part of the "structural protein" of mitochondria is a mixture of membrane carriers.

Another aspect of study is the control of transport by the carrier systems and their interlinkage with the membrane potential, ion gradients, etc. The particularly high potential across the mitochondrial membrane [93] is expected to be particularly effective in such a regulation. It can be assumes that carrier function in its full physiological contect cannot be understood solely in terms of a specific transport but must take into account the control of an unequal distribution of the metabolites across the membrane in accordance with the requirements of cellular metabolism and control.

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