Biochimica et Biophysica Acta, 767 (1984) 369–376
Elsevier

BBA 41632

SUBSTRATE-SITE INTERACTIONS IN THE MEMBRANE-BOUND ADENINE-NUCLEOTIDE CARRIER AS DISCLOSED BY ADP AND ATP ANALOGS

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(Received May 30th, 1984)

Key words: Adenine nucleotide transport; Nucleotide binding; (Bovine heart mitochondria)

(1) The binding parameters of a number of ADP or ATP analogs to the adenine nucleotide carrier in mitochondria and inside-out submitochondrial particles have been explored by means of two specific inhibitors, carboxyatractyloside and bongkrekic acid. (2) The nucleotides tested fell into two classes depending on the shape of the binding curve. Curvilinear Scatchard plots were obtained for the binding of ADP, ATP, adenosine 5′-triphospho-γ-1-(5-sulfonic acid)naphthylamidate ((γ-AmNS)ATP) and adenylyl (β, γ)-methylenediphosphate (p[CH_2]ppA); on the other hand, rectilinear Scatchard plots were obtained in the case of naphthoyl-ADP (N-ADP) and 8-bromo ADP (8Br-ADP) binding. The total number of binding sites for N-ADP and 8Br-ADP could be extrapolated with good accuracy to 1.3–1.5 nmol/mg protein; this value corresponds to the number of carboxyatractyloside-binding sites in heart mitochondria (Block, M.R., Pougeois, R. and Vignais, P.V. (1980) FEBS Lett. 117, 335–340). On the other hand, because of the curvilinearity of the Scatchard plots for the binding of ADP, ATP, (γ-AmNS)ATP and p[CH_2]ppA, the total number of binding sites for these nucleotides could only be approximated to a value higher than 1 nmol/mg protein, the exact value being probably equal to that found for N-ADP and 8Br-ADP binding, i.e. 1.3–1.5 nmol/mg protein. Curvilinearity of Scatchard plots was discussed in terms of negative interactions between nucleotide-binding sites located on the same face of the adenine nucleotide carrier. A possible relationship between the features of the binding plots and the transportable nature of the nucleotide is discussed. (3)Contrary to the enhancing effect of bongkrekic acid on [^{14}C]ADP uptake observed essentially in nucleotide-depleted heart mitochondria (Klingenberg, M., Appel, M., Babel, W. and Aquila, H. (1983) Eur. J. Biochem. 131, 647–654), binding of bongkrekic acid to nondepleted heart mitochondria was found to partially displace previously bound [^{14}C]ADP. These opposite effects of bongkrekic acid may be explained by assuming that bongkrekic acid is able to abolish negative cooperativity between external (cytosolic) ADP-binding sites.

Introduction

The number of nucleotide-binding sites on the mitochondrial adenine nucleotide carrier and the affinity of these sites for ADP and ATP have been routinely explored for many years by a differential method based on the use of atractyloside, a competitive and nonpermeant inhibitor of adenine nucleotide transport which attacks the adenine nucleotide carrier in mitochondria from the outside; atractyloside binding results in the release of the carrier-bound ADP and ATP. The Scatchard plots for the carrier-bound ADP or ATP (ADP or

Abbreviations: (γ-AmNS)ATP, adenosine 5′-triphospho-γ-1-(5-sulfonic acid)naphthylamidate; N-ADP, naphthoyl-ADP; p[CH_2]ppA, adenylyl (β, γ)-methylenediphosphate; 8Br-ADP, 8-Bromo aDP; Mops, 4-morpholinepropanesulfonic acid; Mes, 4-morpholineethanesulfonic acid.

ATP removable by atractyloside) were curvilinear [1–3]. The effect of another specific inhibitor, bongkrekic acid, was also studied. To inhibit adenine nucleotide transport, bongkrekic acid must penetrate the inner mitochondrial membrane and it does so at slighlty acidic pH; it attacks the adenine nucleotide carrier from the inside [4]. Bongkrekic acid added to nucleotide-depleted mitochondria after [^{14}C[ADP enhanced incorporation of [^{14}C]ADP into the mitochondria; this result is the main criterion in favor of a transport mechanism for the adenine nucleotide carrier, referred to as single-site mechanism, in which the adenine nucleotide carrier is supposed to possess a single reorientable nucleotide site exposed alternatively to the inside and the outside [5]. Accumulation of [^{14}C]ADP in the presence of bongkrekic acid was, however, not observed in liver mitochondria, suggesting that the given explanation is not univoqual. Other studies based on the use of fluorescent derivatives of ADP and ATP, namely formycin triphosphate, N-ADP and N-ATP, and performed with mitochondria, submitochondrial particles and the isolated carrier protein strongly suggested the occurrence of interactions between distinct nucleotide-binding sites in the adenine nucleotide carrier [6–8], a conclusion obviously in contrast with the idea of a single reorientable site. Consistent with these later data, kinetic studies have shown that adenine nucleotide transport conforms to a termolecular mechanism, which implies positive interactions between distinct nucleotide-binding sites exposed to the outer and inner face of the membrane-bound carrier [9,10]. In order to get a better insight in the understanding of nucleotide interaction with the adenine nucleotide carrier, we have extended the ADP- and ATP-binding studies to a number of nucleotide analogs in both mitochondria and inside-out submitochondrial particles. Two groups of binding data were obtained depending on the nucleotide used. Some of nucleotides were found to bind to apparently homogeneous sites; for the other nucleotides, the binding was heterogeneous, and in first approximation, it could be ascribed to two classes of sites, the total number of sites being equal in both situations. The implication of these data in the context of interactions between substrate sites on the adenine nucleotide carrier is discussed. The effect of bongkrekic acid on the binding of ADP to intact mitochondria was also examined; its addition resulted in the release of ADP instead of promoting extra binding as reported for nucleotide-depleted mitochondria [5]. The paradoxical effects of bongkrekic acid are rationalized by postulating that bongkrekic acid abolishes interactions between external (cytosolic) nucleotide sites on the adenine nucleotide carrier.

Materials and Methods

Materials. Carboxyatractyloside was purchased from Boehringer and bongkrekic acid was prepared as in Ref. 4. [^{3}H]ATP and ^{3}H-labeled p[CH_2]ppA were obtained from Amersham, and [^{14}C]ADP from New England Nuclear. [^{14}C]8Br-ADP was synthetised as in Ref. 11 and purified by chromatography on a DEAE-cellulose column (DE-52 Whatman), using a 0.05–0.4 M linear gradient of triethanolamine-carbonate buffer (pH 8). Synthesis and purification of [^{14}C](γ-AmNS)ATP and that of [^{14}C]N-ADP are described in Ref. 6 and 7, respectively.

Subcellular preparations. Bovine heart mitochondria were prepared as in Ref. 13 and stored in liquid nitrogen. Inside-out submitochondrial particles were obtained by differential centrifugation after ultrasonic irradiation of bovine heart mitochondria [14,15]. Prior to use, the particles were pretreated with 10 μM carboxyatractyloside, to avoid interference of leaky vesicles or residual right-side-out particles having their carboxyatractyloside-binding sites exposed to the outside.

Binding assays. Binding of radiolabeled nucleotides to the nucleotide sites of the adenine nucleotide carrier (specific binding) in mitochondria was assessed by the inhibitor chase procedure [1], except that atractyloside was replaced by carboxyatractyloside. Mitochondria were suspended at the final concentration of 2 mg protein/ml in 120 mM KCl/10 mM Mops/1 mM EDTA/0.1 mM EGTA, final pH 7.2. 1-ml samples of this suspension were distributed into centrifuge tubes together with increasing concentrations of the nucleotide tested. Incubation was for 30–40 min at 0–2°C. These conditions were required for full isotopic equilibrium. Then, the suspensions in every tube were divided in two parts. One was mixed with 10

μM carboxyatractyloside and incubation was pursued for 30 min at 0–2°C for complete release of specifically bound nucleotides [16]. The other part was kept as control. The incubation was terminated by centrifugation and the pellet was washed twice with unlabeled incubation medium, before being solubilized with a mixture of 5% Triton X-100/0.5 M NaCl. The radioactivity was determined by liquid scintillation. The difference between the radioactivity incorporated into untreated and carboxyatractyloside-treated mitochondria was ascribed to specific nucleotide binding. To assess specific nucleotide binding to inside-out submitochondrial particles, the same procedure was adopted except that bongkrekic acid was used as releasing reagent instead of carboxyatractyloside.

Determination of binding parameters. The K_d values and the total number of nucleotide-binding sites were readily calculated in the case of saturable binding to a single type of sites, a situation which is illustrated by linear Scatchard plots. On the other hand, when the Scatchard plots were curvilinear, they were arbitrarily fitted with a model in which each functional units contains two sites. In this model, the following alternative are possible. (1) The sites of each class do not interact; they differ by their K_d values. (2) All sites are equivalent in the absence of nucleotide; when titration begins, as soon as one nucleotide molecule is bound to any of the sites, the binding of another nucleotide molecule at another site is altered; for example, binding of high affinity at the first site will result in binding of lower affinity at the second site by a mechanism of negative cooperativity.

Assuming two noninteracting sites, the number of bound nucleotides N_B at a given concentration of free nucleotide, N_F, is related to the total number of sites S and the K_d values, K_{d1} and K_{d2} (high and low affinities) by the equation:

$$N_B = S\left(\frac{N_F}{K_{d1} + N_F} + \frac{N_F}{K_{d2} + N_F}\right)$$

On the other hand, assuming two interacting sites which a priori are not different, it is postulated that occupancy of either site by the first nucleotide occurs with high affinity (K_{d1}) and induces a conformational change of the second site resulting in low-affinity binding of the second nucleotide (K_{d2}). Under these conditions, the number of bound nucleotides is related to N_F, K_{d1} and K_{d2} by the Adair's equation:

$$N_B = \frac{S}{2}\left(\frac{N_F/K_{d1} + 2N_F^2/K_{d1}\cdot K_{d2}}{1 + N_F/K_{d1} + N_F^2/K_{d1}\cdot K_{d2}}\right)$$

Results

Binding parameters for adenine nucleotides and their analogs in the case of mitochondria

The data of Fig. 1A concerning the binding of [^{14}C]ADP to the adenine nucleotide carrier in bovine heart mitochondria were obtained by a differential method based on the use of carboxyatractyloside; the specifically bound [^{14}C] ADP was released upon incubation with carboxyatractyloside (Materials and Methods). Curvilinear Scatchard plots were obtained. Similar results were obtained for [^{14}C]ATP (not shown). For ^{3}H-labeled p[CH_2]ppA, an analog which is transported but not metabolized, and for [^{14}C] (γ-AmNS)ATP, another analog which is not transported, the Scatchard plots were also curvilinear (Fig. 1B and C). As explained in Materials and Methods, two models could fit the experimental data, a model with two distinct non-interacting sites and another with two interacting sites; in both cases, the sites differred by affinity. To assess the K_d values of the high and low affinity for ADP, ATP, p[CH_2]ppA and (γ-AmNS)ATP, the binding data were plotted in a semi-log graph (not shown). Fitting of the experimental data to theoretical curves for noninteracting and interacting sites (cf. Materials and Methods) did not allow us to choose between these alternatives. In contrast with the above nucleotides, N-ADP and 8Br-ADP appeared to bind to homogeneous sites, the total number of sites amounting to 1.3–1.5 nmol/mg protein (Fig. 2 and Table I). The binding affinity for N-ADP (K_d 3 μM) was however much higher than for 8Br-ADP (K_d 190 μM).

Due to the curvilinearity of the plots, an accurate determination of the total number of binding sites for ADP, ATP, p[CH_2]ppA and (γ-AmNS)-ATP was not feasible: from extrapolation of the

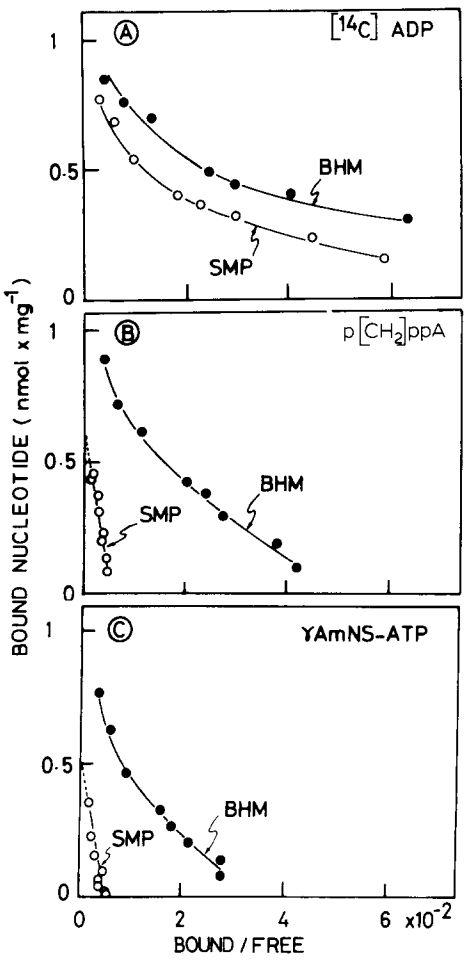

Fig. 1. Curvilinear Scatchard plots of nucleotide binding on mitochondria (BHM) and inside-out submitochndrial particles (SMP). Binding assays were performed as described under Materials and Methods with [^{14}C]ADP. ^{3}H-labeled p[CH_2]ppA or [^{14}C](γ-AmNS)ATP added at concentrations ranging between 2.5 and 200 μM.

Schatchard plot, it was however clear that this number was at least at high as 1 nmol/mg protein and possibly equal to the total number of binding sites for 8Br-ADP and N-ADP, i.e., 1.3–1.5 nmol/mg protein. By fitting the curvilinear Scatchard plots with a two site-model (cf. Materials and Metods), the number of high-affinity sites for ADP, ATP, p[CH_2]ppA and (γ-AmNS)ATP was lower than 0.7 nmol/mg protein; it could be safely approximated to 0.6 ± 0.1 nmol/mg protein.

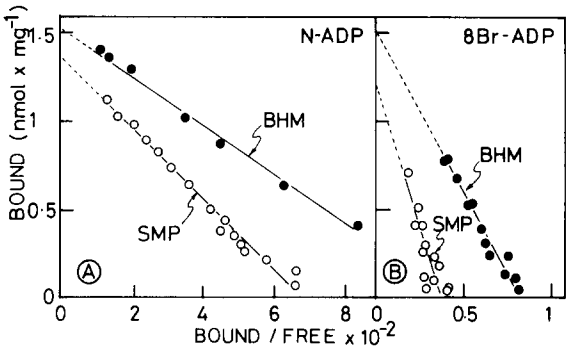

Fig. 2. Fig. 2. Linear Scatchard plots of nucleotide binding on mitochondria (BHM) and inside-out submitochondrial particles (SMP). Binding assays were performed as described under Materials and Methods with [^{14}C]N-ADP or [^{14}C]8Br-ADP at concentrations ranging between 2.5 and 400 μM.

Binding parameters for adenine nucleotides and their analogs in the case of inside-out submitochondrial particles

To eliminate binding artefacts due to leaky or right-side-out particles (see Materials and Metods), binding of nucleotides to inside-out submitochondrial particles was assayed after pretreatment of the particles with 10 μM carboxyatractyloside. This treatment allowed determination of the actual K_d values of the matrix binding sites. The total number of sites was, however, underestimated due to the fact that the inside-out and nonleaky particles corresponded to a fraction only (60–70%) of the total population of submitochondrial particles. However, since this fraction was roughly constant in routine preparations, comparison of the relative number of binding sites for the different nucleotides tested was warranted. As in mitochondria, the binding sites for N-ADP and 8Br-ADP in inside-out particles were apparently homogeneous. On the contrary, curvilinear plots were obtained for ADP and ATP binding, pointing to heterogeneous sites. Assuming an equal number of high- and low-affinity sites, it could be estimated that the total number of specific N-ADP- and 8Br-ADP-binding sites in inside-out particles (Fig. 2) was roughly twice as high as the number of high-affinity sites for ADP and ATP (Fig. 1A and Table I), and approximately equal to the total number of sites for ADP or ATP (high- and low-affinity binding sites), a situation comparable to that encountered with intact mitochondria. The

TABLE I

BINDING PARAMETERS OF NUCLEOTIDES AND NUCLEOTIDE ANALOGS ON THE ADP/ATP CARRIER PROTEIN

K_d values were calculated according to the mathematical treatment described under Materials and Methods, n.d., not determined.

Nucleotide	Bovine heart mitochondria				Submitochondrial particles			
	K_{d1} (μM)	K_{d2} (μM)	Number of sites (nmol/mg)	Transport	K_{d1} (μM)	K_{d2} (μM)	Number of sites (nmol/mg)	Transport
ATP	7	180	≥ 1.0	+	4	75	≥ 0.9	+
ADP	4	65	≥ 1.0	+	6	83	0.9	+
p[CH_2]ppA	10	150	≥ 1.0	+	128	n.d.	n.d.	±
(γ-AmNS)ATP	18	310	≥ 1.0	–	70	n.d.	n.d.	–
N-ADP	3	–	1.3–1.5	–	5.5	–	1.2–1.4	–
8Br-ADP	190	–	1.3–1.5	–	300	–	1.1–1.3	–

affinity of ^{3}H-labeled p[CH_2]ppA and [^{14}C](γ-AmNS)ATP for inside-out particles was too low for an accurate determination of binding parameters.

Effect of bongkrekic acid on binding of [^{14}C]ADP to heart mitochondria

It has been reported [5] that nucleotide-depleted heart mitochondria incubated with [^{14}C]ADP are able to incorporate more [^{14}C]ADP when the medium is supplemented with bongkrekic acid than in the absence of bongkrekic acid. Conversely, addition of ADP or ATP enhanced the binding capacity of [^{3}H]bongkrekic acid [4]. Synergism between ADP and bongkrekic acid was not found, however, for binding to liver mitochondria. As shown in Fig. 3, in the case of nondepleted heart mitochondria, bongkrekic acid, instead of enhancing [^{14}C]ADP uptake, induced partial release of the previously bound [^{14}C]ADP. The Scatchard plot of the difference between the amount of [^{14}C]ADP bound in the absence and in the presence of bongkrekic acid was linear. The total number of nucleotide-binding sites, sensitive to bongkrekic acid, amounted to 0.7 nmol/mg protein, and the apparent K_d value relative to these sites was 47 μM (Fig. 3). Due to the specific interaction of bongkrekic acid with the adenine nucleotide carrier, the released [^{14}C]ADP was most probably bound, before release, to the adenine nucleotide carrier.

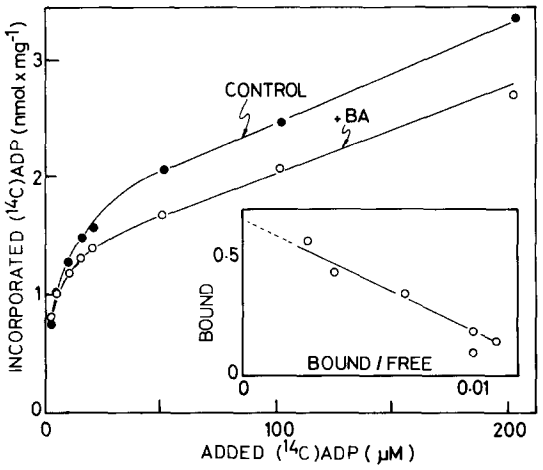

Fig. 3. Effect of bongkrekic acid on [^{14}C]ADP binding on mitochondria. Binding assays were performed as described under Materials and Methods with [^{14}C]ADP at concentrations ranging between 2.5 and 200 μM. The standard saline medium (pH 7.2) was replaced by a buffer made of 120 mM KCl/10 mM Mes (pH 6.5)/1 mM EDTA/0.1 mM EGTA, acidic pH being required for the accesss of bongkrekic acid to its target site.

Discussion

Localization of the nucleotide-binding sites

The termolecular mechanism proposed for ADP/ATP transport [9,10] implies strong positive cooperativity in the binding of adenine nucleotides on the inner and outer faces of the carrier. The bound nucleotides that are displaced by carboxyatractyloside and bongkrekic acid are likely to occupy sites of the carrier exposed either to the outside, or to both the outside and the inside. With

unleaky vesicles, nucleotides released from the inner face of the carrier to the inside cannot be measured; if the vesicles are leaky, those nucleotides escape to the outside and are expected to be released in a time-dependent manner. In the case of [^{14}C]ADP-loaded mitochondria, the amount of [^{14}C]ADP released upon addition of carboxyatractyloside was maximal at 1 min and did not vary for periods of time up to 45 min (not shown), which suggests that the released nucleotides were those initially bound to the outer face of the carrier. The same observation holds for the inside-out particles loaded with [^{14}C]ADP, and then treated with bongkrekic acid. A similar conclusion applied to transportable nucleotides like ATP and p[CH_2]ppA, and nontransportable nucleotides like (γ-AmNS)ATP, 8Br-ADP and N-ADP.

Significance of the low-affinity nucleotide binding

Because of the high specificity of carboxyatractyloside and bongkrekic acid for adenine nucleotide transport, the low-affinity K_d value found for some of the nucleotides tested has probably to be ascribed to a specific interaction with the membrane-bound carrier. In other words, the curvilinear Scatchard plots found for a number of nucleotides are most likey the result of specific nucleotide-carrier interactions. Alternatively, to explain the low-affinity binding, the following hypotheses can be formulated. (1) Intramitochondrial nucleotide compete with externally added nucleotide for binding to the adenine nucleotide carrier on the matrix side. (2) Added nucleotides are metabolized into species which have lost binding affinity, (3) Excess of added nucleotide results in binding inhibition. Curvilinear Scatchard plots were observed with added nucleotides that are not transported and vesicles that are not leaky, which rules out the first hypothesis; they were also observed with nucleotides that are not metabolized like p[CH_2]ppA and (γ-AmNS)ATP; this result invalidates the second hypothesis. Finally, should inhibition by excess substrate be the cause of the curvilinear Scatchard plots, this would also hold for N-ADP and 8Br-ADP; this is not the case.

The curvilinear Scatchard plots for nucleotide binding can therefore be reasonably explained by negative interactions between two specific binding sites located on the same face of the membrane, either the cytosolic face in the case of intact mitochondria or the matrix face in the case of inside-out submitochondrial particles. Four nucleotide sites per functional carrier unit have also been proposed on the basis of fluorescence data obtained with formycin 5′-triphosphate, a transportable analog of ATP [8]. Negative interactions between two external nucleotide sides or between two internal nucleotide sites as suggested by binding studies, and positive interactions between external and internal sites as postulated on the basis of kinetic studies [9–10] are illustrated in the scheme of Fig. 4.

One may wonder why binding of ADP, ATP, p[CH_2]ppA and (γ-AmNS)ATP exhibits negative cooperativity whereas one set of equivalent binding sites is found for N-ADP and Br-ADP. Since binding of a nucleotide to the carrier is the prerequisite step for transport, the explanation might reside in the fact that the binding features depend on the transportable or nontransportable nature of the nucleotide. Binding followed by transport would be accompanied, at the binding step, by conformational changes of the carrier, resulting in negative cooperativity; this is the case of ADP, ATP and p[CH_2]ppA. Binding without transport which is typical of N-ADP and Br-ADP would correspond to one set of equivalent binding sites. The negative cooperativity exhibited by the binding of (γ-AmNS)ATP, (which binds but is not transported) might represent the intermediate case of a nucleotide whose binding to the carrier triggers conformational changes of the carrier, without achievement of the final step of transport.

Why does bongkrekic acid either enhances or decreases nucleotide binding?

As shown in Fig. 3, bongkrekic acid induces release of carrier-bound [^{14}C]ADP. The difference between bound [^{14}C]ADP in the absence and presence of bongkrekic acid led to linear Scatchard plots, indicating that the released nucleotides were previously bound to a single type of sites with a K_d of 47 μM, which corresponds to a medium affinity. This result contrasts with data of other experiments carried out with depleted heart mitochondria showing that bongkrekic acid increases the number of bound nucleotides [5]. To explain that addition of bongkrekic acid may lead

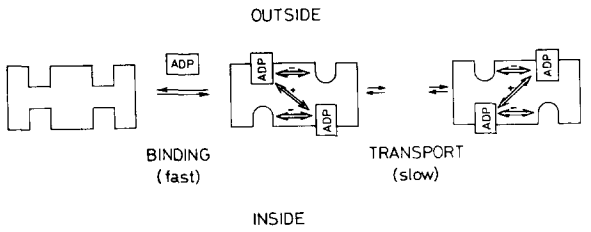

Fig. 4. Scheme illustrating interactions between nucleotide-binding sites within the functional carrier unit (for detail, see text).

either to a decrease of bound ADP or to an excess binding of ADP, it is postulated that binding of added ADP is governed by a phenomenon of negative cooperativity between two nucleotide sites located on the cytosolic side of the carrier and that bongkrekic acid abolished this negative cooperativity upon binding to the other side. In other words, at low concentration, ADP binds with high affinity (K_{d1}) to the first site; this results in a decreased affinity of the second site which can be filled only at higher concentrations of ADP, corresponding to an apparent K_{d2} value. This negative cooperativity is illustrated by the Adair's equation:

$$ADP_{bound} = \frac{S}{2}\left(\frac{ADP_{free}/K_{d1} + 2(ADP_{free})^2/K_{d1}\,K_{d2}}{1 + ADP_{free}/K_{d1} + (ADP_{free})^2/K_{d1}\,K_{d2}}\right)$$

where S is the total number of binding sites. In the presence of bongkrekic acid, negative cooperativity between the nucleotide-binding sites is abolished, and the sites bind ADP with the same affinity corresponding to the new K_d value, K_{d3}. Under these conditions, ADP binding obeys the following relationship:

$$ADP_{bound} = S\left(\frac{ADP_{free}}{K_{d3} + ADP_{free}}\right)$$

Based on this model, theoretical binding curves were obtained, using the following values: $S = 1.2$ nmol/mg protein, $K_{d1} = 4\ \mu M$, $K_{d2} = 65\ \mu M$ and K_{d3} ranging between 4 and 100 μM. The values chosen for K_{d1} and k_{d2} were those obtained in displacement assays of bound [^{14}C]ADP by carboxyatractyloside. As shown in Fig. 5, the control curve D (without bongkrekic acid) characterized by two K_d values, K_{d1} and K_{d2}, was located below the curves obtained for a single affinity binding site with K_{d3} values lower than 16 μM (curves A, B and C) and above the curves corresponding to a single affinity binding site with K_{d3} values higher than 32 μM (E, F and G). Thus, varying the values of K_{d1}, K_{d2} and K_{d3} (depending on biological preparations) results either in an increase or a decrease of the apparent ADP-binding capacity at nonsaturable concentrations of ADP.

Hypothesis on the degree of oligomerization of the membrane-bound ADP / ATP carrier based on ligand stoichiometry

The carboxyatractyloside-protein complex solubilized in nonionic detergents such as Triton X-100 and lauryl aminopropylaminoxide is a dimer [17,18]. 1 mol carboxyatractyloside binds with high affinity to 1 mol of carrier dimer [19,20]. The total number of carboxyatractyloside-binding sites in mitochondria amounts to 1.2–1.5 nmol/mg protein [16]. This value is roughly twice higher than the number of the high-affinity sites for ADP, ATP, p[CH_2]ppA and (γ-AmNS)ATP determined in the present work. On the other hand, it is equal to the number of sites at saturation found for the binding of 8Br-ADP and N-ADP. One is therefore faced with the following paradox. Each high-affinity site for ADP, ATP, p[CH_2]ppA and (γ-AmNS)ATP corresponds to two high-affinity carboxyatractyloside-binding sites. Since there is one high-affinity carboxyatractyloside-binding site per carrier dimer, one is led to conclude that there is one high-affinity nucleotide-binding site per couple of carrier dimers. In other words, if transport is preceded by high-affinity binding of the transported nucleotide as probably it is, the functional ADP/ATP carrier would have to assume a tetrameric structure. Inhibition of adenine nucleotide transport by carboxyatractyloside and bongkrekic acid could be the consequence of the dissociation of this tetrameric structure into individual dimers. It should be understood that this hypothesis stems from the contention that each mol of carrier dimer binds 1 mol carboxyatractyloside [19,20] and is not yet supported by structural data; therefore, it is proposed essentially as a working hypothesis to explain paradoxical binding features of the ADP/ATP carrier protein relative to carboxyatractyloside and nucleotides.

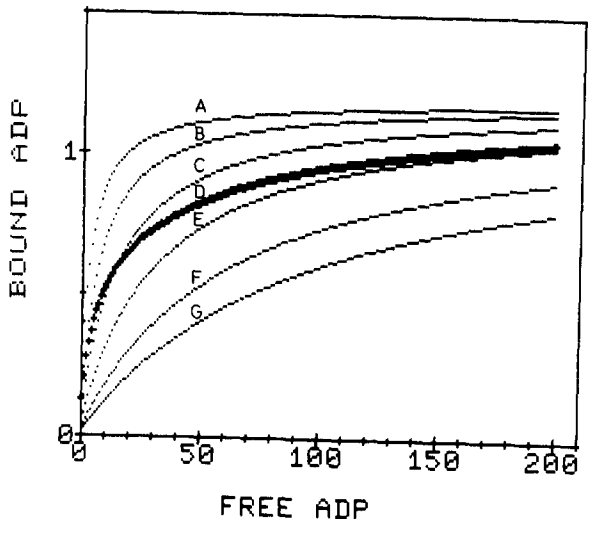

Fig. 5. Theoretical ADP-curves in the presence and absence of bongkrekic acid. The theoretical treatment was based on two negatively interacting sites in the absence of bongkrekic acid, and abolition of negative interactions by bongkrekic acid. Curve D (ADP binding in the absence of bongkrekic acid) corresponds to the Adair's equation (see Discussion):

$$\text{ADP bound} = \frac{S}{2}\left(\frac{\text{ADP}_{\text{free}}/K_{\text{d1}} + 2(\text{ADP}_{\text{free}})^2/K_{\text{d1}}\,K_{\text{d2}}}{1 + \text{ADP}_{\text{free}}/K_{\text{d1}} + (\text{ADP}_{\text{free}})^2/K_{\text{d1}}\,K_{\text{d2}}}\right)$$

The other traces (binding in the presence of bongkrekic acid) correspond to the expression:

$$\text{ADP}_{\text{bound}} = S \times \frac{\text{ADP}_{\text{free}}}{K_{\text{d3}} + \text{ADP}_{\text{free}}}$$

Curve D was drawn using K_{d1} equal to 4 μM and K_{d2} equal to 65 μM (see Table I). S was taken equal to 1.2 nmol/mg protein. Curves A, B, C, E, F and G were obtained with K_{d3} values of 4, 8, 16, 32, 64 and 100 μM, respectively.

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