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Quantitative analysis of the 'phosphocreatine shuttle': I. A probability approach to the description of phosphocreatine production in the coupled creatine kinase-ATP/ADP translocase-oxidative phosphorylation reactions in heart mitochondria

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For the first time, a probability approach was used to describe heart mitochondrial respiration in the medium with ATP, Cr and PCr but without ADP Respiring mitochondria were considered as a three-component system, including (1) oxidative phosphorylation reactions which provide stable ATP concentration in the mitochondrial matrix, (2) adenine nucleotide translocase, which provides exchange transfer of matrix ATP for outside creatine kinase-supplied ADP when both substrates are simultaneously bound to translocase and (3) creatine kinase, starting these reactions when activated by the substrates from medium The specific feature of this system is a close proximity of creatine kinase and translocase molecules This results in high probability of direct activation of translocase by creatine kinase-derived ADP without its leak into the medium In turn, the activated translocase with the same high probability directly provides creatine kinase with matrix-derived ATP The catalytic complexes of creatine kinase with ATP from matrix together with those formed from substrates from medium provide high activation of creatine kinase coupled to translocase activation The considered probabilities were arranged into a mathematical model The model satisfactorily simulates the experimental data by Jacobus, W E and Saks, V A ((1982) Arch Biochem Biophys 219, 167–178), who investigated this system in all regimens of functioning The results suggest the observed kinetic and thermodynamic irregularities in the behavior of structurally-bound creatine kinase as a direct consequence of its tight coupling to translocase

Introduction

Detailed kinetic analysis of the creatine kinase reaction catalyzed by soluble enzymes has been given many years ago [1–3] However, it has been experimentally established during last two decades that in cardiomyocytes, skeletal muscle cells and also in the brain and other specific types of cells creatine kinase is specifically compartmentized [4–6] For example, in heart cells only half of the enzymes is found to be localized in cytoplasm and extracted with soluble fraction of enzymes, but another half of cellular activity is represented by specific isoenzymes bound to mitochondrial membrane, to the cellular membranes and to myofibrillar structures [6] The behavior of the structurally-bound enzymes is controlled by and highly dependent on its microenvironment, mostly by the surrounding enzymes In mitochondria, creatine kinase is controlled by adenine nucleotide translocase and in other structures it is controlled by ATPases [4,5] It has already been known since 1975 [3] that the behavior of the creatine kinase in mitochondria under conditions of oxidative phosphorylation is not governed by substrate concentration in the medium and soluble enzyme kinetics, but even the direction of the creatine kinase reaction may be different depending of the oxidative phosphorylation which very significantly increases the rate of phosphocreatine production and decreases the rate of the reversed reaction of ATP formation The control of oxidative phosphorylation over mitochondrial creatine kinase reaction has been shown both kinetically and thermodynamically [3] Kinetically, it is manifested as a specific change in the dissociation

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constant for MgATP from the ternary enzyme-substrate complexes [7,8], and thermodynamically it is manifested as a maintenance of the reaction in the direction opposite to that predicted from mass action ratio in the medium and equilibrium constant value [3,9] All that evidence was taken to show the functional coupling between mitochondrial creatine kinase and adenine nucleotide translocase translocase directs ATP molecules directly to the active site on creatine kinase and simultaneously removes the reaction product-ADP [2–12] Such a close interaction is based on the close proximity of the enzymes creatine kinase is bound to the cardiolipin molecules closely surrounding adenine nucleotide translocase in the inner mitochondrial membrane, thus forming a transport protein-enzyme complex [13,14] However, the functioning of this complex has not yet described quantitatively in sufficient details only short and rather general model has been published in 1976 [15] It is the aim of this work to develop a mathematical model quantitatively describing the process of aerobic phosphocreatine synthesis coupled to oxidative phosphorylation in cardiac mitochondria

Model

Mathematical model of creatine kinase reaction coupled to adenine nucleotide translocation and oxidative phosphorylation

The model is based on a probability approach to the description of the enzyme-enzyme interaction The first part of model is a kinetic equation of the creatine kinase reaction itself, which is then incorporated into a more general model

I The mitochondrial creatine kinase reaction

The creatine kinase reaction mechanism is of rapid-equilibrium random binding Bi-Bi type, according to Cleland's classification [2,16,17] This mechanism is schematically illustrated in Fig 1 In this figure, the dissociation constants, K, of enzyme-substrate complexes are given for primary complexes with an index i (initial), and for ternary complexes only with the symbol for the substrate a (A) for ATP, d (D) for ADP, cr (Cr) for creatine and cp (PCr) for phosphocreatine The central complex for the forward reaction is E A Cr which is converted into that for the reverse reaction E D PCr with the rate constant kk_1 Besides these enzymatically active complexes, there are dead-end complexes E D Cr and with low probability E A PCr Dissociation constants of substrates of these complexes are given with the symbol I (their formation is Inhibitory for the reaction)

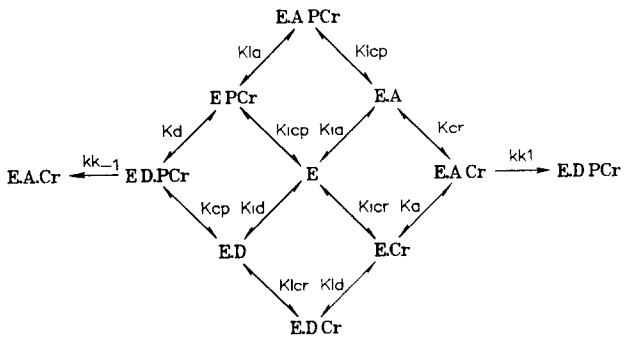

Fig 1 Kinetic scheme of the creatine kinase reaction mechanism Details in the text

According to the rapid equilibrium random binding Bi-Bi-type mechanism the binding and dissociation of substrates and products is very rapid and the reaction rate is determined by interconversion of the ternary complexes [2,16,17] Interconversion of the central ternary complex E A Cr into enzyme-product complex E D PCr occurs with the rate constant kk_1, and the equations for this reaction are given in several earlier works [2,3,15] In the equilibrium or steady state, the distribution of the enzyme between enzyme-substrate complexes and free state can be expressed in terms of probabilities for the purpose of modeling of coupled reactions For example, the probability (P) of the existence of the enzyme in the free state, (E), is given by

$$P(\mathrm{E}) = [\mathrm{E}]/[\mathrm{E}_{tot}]$$

$$= 1/(1 + [\mathrm{Cr}]/K_{icr} + [\mathrm{A}]/K_{ia} + [\mathrm{A}]\times[\mathrm{Cr}]/(K_{ia}\times K_{cr})$$

$$+ [\mathrm{PCr}]/K_{icp} + [\mathrm{PCr}]\times[\mathrm{A}]/(K_{icp}\times K_{ia})) = 1/\mathrm{Den} \quad (1)$$

where E_{tot} designates the total concentration of enzyme and Den designates the denominator

The probabilities of the existence of the enzyme in different enzyme-substrate complexes are given by the following equations

$$P(\mathrm{E\ A}) = [\mathrm{E\ A}]/[\mathrm{E}_{tot}] = [\mathrm{A}]/(K_{ia}\ \mathrm{Den}) \quad (2)$$

$$P(\mathrm{E\ Cr}) = [\mathrm{E\ Cr}]/[\mathrm{E}_{tot}] = [\mathrm{Cr}]/(K_{icr}\ \mathrm{Den}) \quad (3)$$

$$P(\mathrm{E\ A\ Cr}) = [\mathrm{E\ A\ Cr}]/[\mathrm{E}_{tot}] = [\mathrm{A}]\ [\mathrm{Cr}]/(K_{ia}\ K_{cr}\ \mathrm{Den}) \quad (4)$$

$$P(\mathrm{E\ PCr}) = [\mathrm{E\ PCr}]/[\mathrm{E}_{tot}] = [\mathrm{PCr}]/(K_{icp}\ \mathrm{Den}) \quad (5)$$

$$P(\mathrm{E\ A\ PCr}) = [\mathrm{E\ A\ PCr}]/[\mathrm{E}_{tot}] = [\mathrm{PCr}]\ [\mathrm{A}]/(K_{icp}\ K_{ia}\ \mathrm{Den}) \quad (6)$$

The effective concentration of each complex is expressed as a product of its probability and the total concentration of the enzyme, E_{tot} The rate of reaction product formation, ADP and PCr, in the forward reaction is given by the following equation

$$V1 = E_{tot}\ P(\mathrm{E\ A\ Cr})\ kk_1 \quad (7)$$

This routine consideration is valid for the soluble creatine kinase [2] In the coupled system the creatine

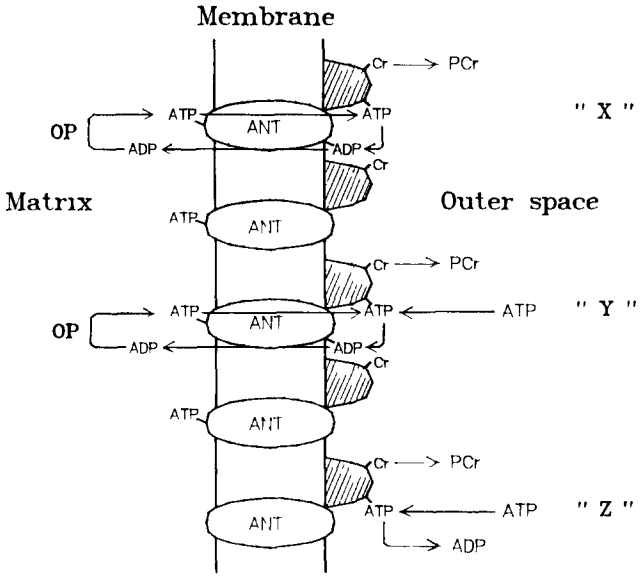

Fig 2 Schematic representation of three possible combinations of sources of ATP for mitochondrial phosphocreatine production In the energized state, a significant population of adenine nucleotide translocase (ANT) molecules bind ATP from mitochondrial matrix and in the presence of catalytic amounts of ADP from mitochondrial creatine kinase, CK_{mit} (shaded areas), transports ATP across the membrane directly to the active site of CK_{mit} In the pathway X, this mitochondrial ATP is a single source of ATP for the creatine kinase reaction Besides this, ATP can also bind from the surrounding medium, which is the second possible source for ATP for CK_{mit} Phosphocreatine production from this source is illustrated as the pathway Y Both in X and Y pathways ADP, produced simultaneously with phosphocreatine, PCr, is preferentially directed back to translocase because of close spatial proximity of CK_{mit} to ANT Finally, in the absence of the oxidative phosphorylation (OP), phosphocreatine is produced solely from ATP from the surrounding medium as shown in the pathway Z

kinase reaction is governed by interaction with other mitochondrial systems, adenine nucleotide translocase and oxidative phosphorylation [3,7–15]

II The creatine kinase reaction coupled to adenine nucleotide translocase and oxidative phosphorylation

It is well-established that the mitochondrial creatine kinase, CK_{mit}, is attached to the outer surface of the inner mitochondrial membrane by electrostatic interactions between cardiolipin of the membrane and lysine residues of the protein [13–14] It is supposed that the creatine kinase is located in the vicinity of adenine nucleotide translocase (ANT) and the latter influences the creatine kinase reaction via changing the effective local concentration of substrates by a mechanism close to the direct channeling (see Fig 2) If the processes of oxidative phosphorylation and adenine nucleotide translocation are activated, the CK_{mit} may produce phosphocreatine and ADP in three different ways depending on the source of ATP Schematically, these processes are illustrated in Fig 2 In the state X in this figure phosphocreatine is produced exclusively from mitochondrial ATP produced in oxidative phosphorylation In the state Y PCr is produced from ATP in the solution, but the ADP formed is directed to translocase In the state Z PCr is exclusively produced from ATP in the medium and the ADP formed is released into medium Eqn 7, given above, describes the enzyme reaction only in the state Z The presence of other processes and enzyme functioning in states X and Y can be accounted for in the following way

Let us consider the fate of ADP after its formation at the active site of mitochondrial creatine kinase in enzyme-substrate complex E A Cr This catalytically effective complex of CK may have been formed both from mitochondrial ATP in the pathway X and ATP in the medium in the pathways Y and Z with total probability $P(CK)_{ef}$ In the intermembrane space of mitochondria where CK_{mit} is located in the close vicinity of ADP/ATP translocase, T, ADP released from the CK_{mit} active center has a high probability to meet the ADP-binding site of translocase at the outer surface of the inner mitochondrial membrane, because of the short diffusion distance This probability may be designated as $P(T_{Dloc})_{out}$ Since there is a basis to believe that in heart mitochondria the number of molecules (monomers with substrate binding sites) of CK_{mit} and T are equal [18], the probability of the formation of the complex of CK_{mit}-derived local ADP with translocase from outside, $P(T\,Dloc)_{out}$, may be expressed as a product of two probabilities, $P(CK)_{ef}$ and $P(T_{Dloc})_{out}$

$$P(T\,Dloc)_{out} = P(CK)_{ef}\ P(T_{Dloc})_{out} \quad (8)$$

PCr does not bind to T, and therefore, may not be considered

ADP, which is bound to translocase from outside, may be transported into mitochondria only in exchange to matrix ATP [19] Therefore, if the probability of binding of ATP from the matrix side to translocase is $P(T\,A)_{in}$, the probability (frequency) of formation of the effective complex of T with both its substrates for their subsequent transfer across the membrane, $P(T)_{ef}$, will be

$$P(T)_{ef} = P(T\,Dloc)_{out}\ P(T\,A)_{in} = P(CK)_{ef}\ P(T_{Dloc})_{out}\ P(T\,A)_{in} \quad (9)$$

Activation of T and the translocation of adenine nucleotides results in the replacement of ADP by ATP in the microcompartment between T and CK_{mit} at the outer surface of inner mitochondrial membrane Molecules of ATP which dissociate from T have (as it was for ADP before) rather high probability, because of decreased diffusion distance, to meet with the active center of creatine kinase at the membrane This probability, $P(CK_{Aloc})$, multiplied by probability of local ATP generation by translocase, $P(T)_{ef}$, gives the gen-

eral probability of complex formation between CK_{mit} and matrix-derived local ATP, P(CK Aloc)

$$P(\text{CK Aloc}) = P(\text{T})_{ef}\ P(\text{CK}_{\text{Aloc}}) \tag{10}$$

At the moment when ATP will be directed from T to the CK_{mit}, the latter can be in the form of any of six complexes (E, E Cr, E ATP, E ATP Cr, E PCr, E ATP PCr), the relative contents of which are expressed by the substrate concentrations in the medium and the values of enzyme-substrate complexes dissociation constants (see Eqns 1–7) However, ATP can bind only to the forms E to produce E ATP, to E Cr to produce effective ternary complex E ATP Cr, and in some extent to E PCr producing non-stabile dead-end complex E ATP PCr Collision of the ATP from T with complexes E ATP, E ATP Cr and E ATP PCr cannot result in the binding of ATP, and it should thus leave the microcompartment by diffusion into the medium This path is shown by Y in Fig 2

In the pathway X, the catalytically effective ternary complexes of CK_{mit} are formed directly from E Cr by binding of only mitochondrial ATP transferred across the membrane by T The probability of their formation, P_1, can be calculated by multiplying the general probability of complex formation of CK_{mit} with mitochondrial or 'local' ATP, P(CK Aloc), and the probability for existence of E Cr complex of CK_{mit} (Eqn 3)

$$P_1 = P(\text{CK Aloc})\ P(\text{E Cr}) \tag{11}$$

In a similar way, one can calculate the probability of E ATP formation from mitochondrial ATP (P_{2a}) as

$$P_{2a} = P(\text{CK Aloc})\ P(\text{E}) \tag{12}$$

Now let us consider in more detail the fate of the central effective ternary complex E A Cr This complex may be converted into an enzyme-product complex with the rate constant kk_1 (see Fig 1) Eqn 11 describes the formation of effective ternary complex when creatine is bound first to the enzyme which then reacts with mitochondrial ATP Another possibility is that ATP from T binds to E first (Eqn 12) with subsequent reaction with creatine In this case it is necessary to determine the ratio between mitochondrial ATP which will be bound to CK_{mit}, and that leaking into the medium by a simple process of diffusion Let us consider in Scheme I, which is a fragment of general scheme in Fig 1, the elementary steps of the reactions connected to the conversion of E Aloc This complex,

$$\text{E} \underset{k_{dif}}{\overset{k_{+1}=[\text{A}]\ K_{a+}}{\underset{k_{-1}}{\rightleftharpoons}}} \text{A} \quad \text{E Aloc} \underset{k_{-2}}{\overset{k_{+2}=[\text{Cr}]\ K_{cr+}}{\rightleftharpoons}} \text{E A Cr} \xrightarrow{kk_1}$$

Scheme I Elementary steps in the conversions of E Aloc, the complex of mitochondrial ATP with free CK_{mit}

$$\text{E PCr} \underset{k_{dif}}{\overset{k_{+3}=[\text{A}]\ K_{a+}}{\underset{k_{-3}}{\rightleftharpoons}}} \text{A} \quad \text{E PCr Aloc} \underset{k_{+4}=[\text{PCr}]\ K_{cp+}}{\overset{k_{-4}}{\rightleftharpoons}} \text{E Aloc}$$

Scheme II Elementary steps in the conversions of E PCr Aloc, the complex of mitochondrial ATP with E PCr

formed from mitochondrial ATP, may dissociate with the rate constant k_{-1} with the subsequent leakage of ATP from intermembrane space by diffusion process, characterized by diffusion constant k_{dif} On the other hand, the complex E Aloc may react with Cr, if the second-order rate constant for this reaction is K_{Cr+}, the pseudo-first-order rate constant $k_{+2} = [\text{Cr}]\ K_{Cr+}$ may be used to describe the conversion of E Aloc into the effective ternary complex E ATP Cr The part of E Aloc which is converted into this complex may be described by a partitioning coefficient P_{c1} [20]

$$P_{c1} = k_{+2}/(k_{+2} + k_{-1}) \tag{13}$$

This partitioning coefficient may be used to calculate the probability, P_2, of effective ternary complex formation from E Aloc by its subsequent reaction with creatine

$$P_2 = P_{2a}\ P_{c1} \tag{14}$$

In a similar way, the formation of the dead-end complex E PCr ATPloc may be described First, PCr may bind to CK_{mit} to form E PCr which then reacts with mitochondrial ATP, and the probability of dead-end complex formation will be

$$P_{3a} = P(\text{CK Aloc})\ P(\text{E PCr}) \tag{15}$$

The complex E PCr ATPloc may dissociate with the rate constant k_{-3} with subsequent diffusion of ATP from the intermembrane space (Scheme II) Alternatively, these dead-end complexes may release PCr with the rate constant k_{-4} and be converted into E Aloc The probability of production of E Aloc from the dead-end complex E PCr Aloc may be described again by a second partitioning coefficient, P_{c2}

$$P_{c2} = k_{-4}/(k_{-4} + k_{-3}) \tag{16}$$

which gives P_{3b} as probability for E Aloc production in this reaction path

$$P_{3b} = P_{3a}\ P_{c2} \tag{17}$$

Taking Eqns 12 and 17 together gives the total probability of E Aloc formation before binding of creatine

$$P(\text{E Aloc})_{tot} = P_{2a} + P_{3b} = P(\text{CK Aloc})\ (P(\text{E}) + P(\text{E Pcr})\ P_{c2}) \tag{18}$$

According to Eqns 12–14, by using a partitioning coefficient P_{c1}, the probability of effective ternary complex E Aloc Cr formation, P_4, from E Aloc in all pathways of its production is

$$P_4 = P(\text{E Aloc})_{tot}\ P_{c1} \tag{19}$$

In the steady state, the total probability of the effective ternary complex formation, $P(CK)_{ef}$, is the sum of probabilities of its formation from the local pools of ATP (P_1 and P_4) in pathway X and from free ATP in the solution in the pathways Y and Z (Fig 2, see Eqn 4) So

$$P(CK)_{ef} = P_1 + P_4 + P(\text{E A Cr}) \tag{20}$$

Introducing into this equation the expressions for P_1 (Eqn 11) and P_4 (Eqns 18 and 19) and substituting P(CK Aloc) by $P(T)_{ef}\ P(CK_{Aloc})$ (Eqn 10) we obtain

$$P(CK)_{ef} = P(\text{E A Cr}) + P(T)_{ef}\ P(CK_{Aloc})\ \{P(\text{E Cr}) + [P(E) + P(\text{E PCr})\ P_{c2}]\ P_{c1}\} \tag{21}$$

This is a balance equation for the CK_{mit} effective ternary complexes Their relationship with the effective complexes of translocase is described by Eqn 9 Introducing into this equation the expression for $P(CK)_{ef}$ from Eqn 21, we can find, after the proper transformations, the following final expression for the effective forms of translocase

$$P(T)_{ef} = P(\text{E A Cr})/\{1/[P(T_{Dloc})_{out}\ P(\text{T A})_{in}] - P(CK_{Aloc})\ [P(\text{E Cr}) + [P(E) + P(\text{E PCr})\ P_{c2}]\ P_{c1}]\} \tag{22}$$

This equation is the main one used for the calculations Included P(E), P(E Cr), P(E A Cr), P(E PCr), P_{c1} and P_{c2} probabilities are to be separately calculated from Eqns 1, 3–5, 13 and 16, respectively, using the parameters indicated in the next section $P(CK)_{ef}$ can be calculated from Eqn 9 simply as

$$P(CK)_{ef} = P(T)_{ef}/(P(T_{Dloc})_{out}\ P(\text{T A})_{in}) \tag{23}$$

Knowing the probabilities of the activation of adenine nucleotide translocase and CK_{mit}, and using the known value of the contents of creatine kinase (N) in mitochondria and the value of kk_1 of E ATP Cr conversion (see Eqn 7), one can calculate the absolute value of the steady-state rate of functioning of translocase, V_t

$$V_t = \text{N}\ kk_1\ P(T)_{ef} \tag{24}$$

and the steady-state rate of phosphocreatine production by coupled creatine kinase

$$V_{PCr} = N\ kk_1\ P(CK)_{ef} \tag{25}$$

III Choice of parameters for modeling

The following parameters were used For the mitochondrial creatine kinase reaction all the data were taken from the Jacobus and Saks paper [7] $K_{ia} = 0\ 75$ mM, $K_{icr} = 26$ mM, $K_{icp} = 1\ 6$ mM, $K_a = 0\ 15$ mM, $K_{cr} = 5\ 2$ mM, $K_{Icp} = 24$ mM $K_{Ia} = 11\ 25$ mM was calculated from the thermodynamic equation $K_{icp}\ K_{Ia} = K_{ia}\ K_{Icp}$ $V1_{max} = N\ kk_1 = 1\ 0$ μmol/min per mg protein

For calculation of k_{-1}, the value of the diffusion-limited association rate constant for ATP, $K_{a+} = 2\ 10^7$ $M^{-1}\ s^{-1}$ [21] was used From that value we found $k_{-1} = 2\ 10^7\ M^{-1}\ s^{-1}\ 7\ 5\ 10^{-4}\ M = 15\ 10^3\ s^{-1}$ For calculation of k_{+2}, the value of the constant was taken to be twice the K_{a+} value, $K_{Cr+} = 4\ 10^7\ M^{-1}\ s^{-1}$, as the PCr molecules diffuse about −2fold faster than larger ATP ones [22] k_{-3} was calculated as $K_{a+}\ K_{Ia} = 2\ 10^7\ M^{-1}\ s^{-1}\ 11\ 25\ 10^{-3}\ M = 225\ 10^3\ s^{-1}$ k_{-4} was calculated as $K_{cp+}\ K_{Icp} = 960\ 10^3\ s^{-1}$

$P(\text{T A})_{in} = 0\ 9$ was found by a method of best approximation to the experimental data (see Results) The value of this parameter was taken to be constant in each particular experiment, as the matrix ATP/ADP ratio has been revealed to be constant, about 4, on stimulation of mitochondrial respiration from 0 to 75% of maximum [23]

The probabilities of creatine kinase derived local ADP to meet with translocase, $P(T_{Dloc})_{out}$, and of translocase derived local ATP to meet with creatine kinase, $P(CK_{Aloc})$, were taken to be equal to 1 0 (concept of direct channeling)

The concentrations of ATP, Cr and PCr were as experimentally used The concentration of ADP was taken to be zero

Results

System with two substrates ATP and creatine

The set of Eqns 1–25 was used to calculate the rate of ATP and phosphocreatine production in mitochondria Using the ATP/O_2 ratio equal to six, the velocities of oxygen uptake in the steady state conditions in the presence of creatine were calculated from the rates of ATP transport obtained according to Eqn 24 These rates were calculated for the experimental condition where in the presence of 25 mM of creatine MgATP concentration in the medium was changed In this case creatine and MgATP initiate PCr and ADP production and ADP subsequently stimulates mitochondrial oxidative phosphorylation It is intuitively clear that the efficiency of regulation of oxygen uptake depends on the coupling between creatine kinase and translocase

In our model the probability of ATP transfer from translocase to creatine kinase was taken one (concept of direct channeling) However, one of the parameters,

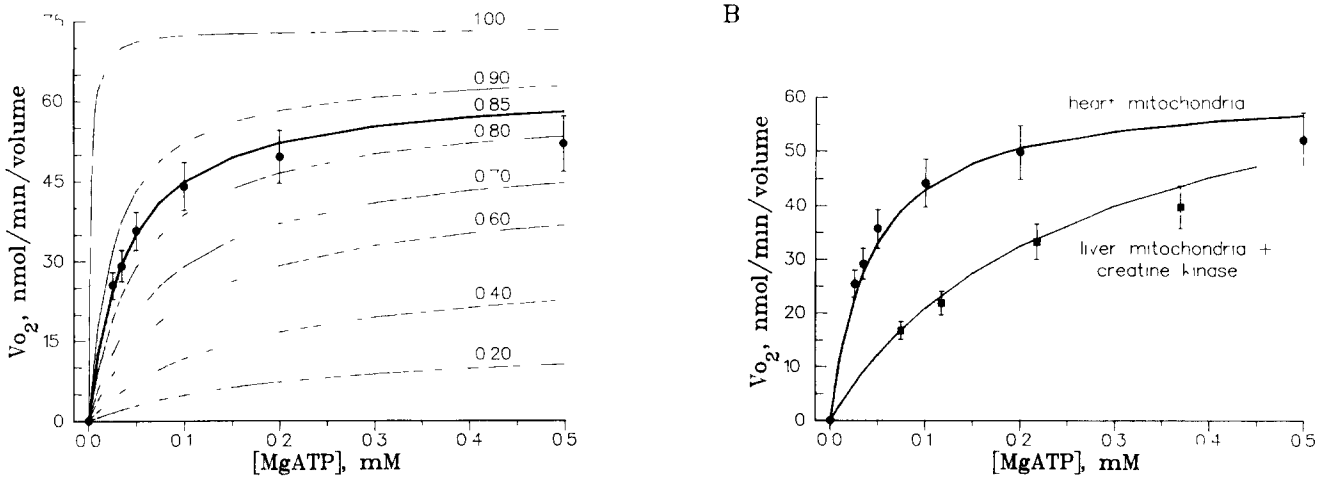

Fig 3 Simulation of ATP-dependence of creatine-kinase-mediated mitochondrial respiration in the presence of 25 mM creatine The experimental data are taken from Fig 44 of Saks [24] They are shown by separate dots with bars showing standard deviation Oxygen consumption rates were expressed per volume of polarographic cell, 1 4 ml, an endogenous state 2 respiration rate being subtracted (A) Fitting of experimental and calculated data with the purpose of the estimation of the value of the probability of matrix ATP binding with translocase, $P(T A)_{in}$ Oxygraph measurements were conducted at 30°C in medium containing 0 25 M sucrose, 10 mM Hepes (pH 7 4), 5 mM K^+ phosphate, 5 mM K^+ glutamate, 2 mM K^+ malate, 3 3 mM $MgCl_2$, 0 3 mM dithiothreitol, 1 0 mg/ml bovine serum albumin, 25 mM creatine, 0–0 5 mM ATP and 0 6 mg rat heart mitochondrial protein No ADP was added An activity of translocase was calculated by Eqn 24 Simulation parameters are given in the Model section, the employed probability ($P(T A)_{in}$) values are indicated above the simulated curves The maximal rate of PCr production by CK_{mit} is 536 nmol/min per 1 4 ml of reaction mixture The rates of oxygen consumption were calculated as the rate of ATP translocation by translocase, divided by ATP/O_2 ratio equal to 6 (B) Simulation of the creatine kinase-controlled respiration in heart mitochondria (upper curve) and liver mitochondria (lower curve) Oxygen consumption by rat heart mitochondria (A) was simulated at an optimal $P(T A)_{in} = 0 835$ The cell with rat liver mitochondria (2 5 mg of protein) was additionally supplied with CK_{mit}, extracted from rat heart mitochondria The total activity of added CK_{mit} was equal to that contained in 0 6 mg of rat heart mitochondria protein Experimental conditions as in (A), the data were simulated by Eqn 7, the rates of PCr production being taken as the rates of ATP translocation

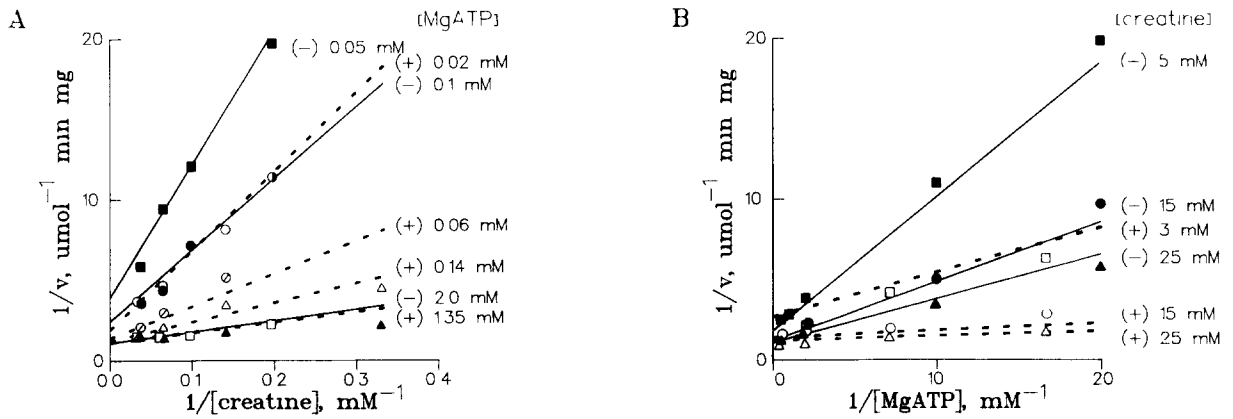

Fig 4 Simulation of the primary double-reciprocal plots of the forward creatine kinase reaction in the presence (+) and absence (−) of oxidative phosphorylation Experimental points are from Fig 2A,B of Jacobus and Saks [7], empty and filled marks corresponding to data obtained in the presence (+) and absence (−) of oxidative phosphorylation, respectively Simulations are presented by thin (−) and thick interrupted (+) lines (A) The dependences of the creatine kinase reaction rates on the concentrations of creatine at fixed initial concentrations of ATP ATP concentrations are indicated at the right side of the figure They are 0 02 (○), 0 06 (Ø), 0 14 (△), 1 35 (□) mM in the presence and 0 05 (■), 0 1 (●) and 2 0 (▲) mM in the absence of oxidative phosphorylation (B) The dependence of the creatine kinase reaction rates on the concentrations of ATP at fixed initial concentrations of creatine Creatine concentrations are indicated at the right They are 3 (□), 15 (○), 25 (△) mM in the presence and 5 (■), 15 (●) and 25 (▲) mM in the absence of oxidative phosphorylation Experimental conditions 0 25 M sucrose, 10 mM Hepes (pH 7 4), 30°C, 0 2 mM EDTA, 5 mM K^+ phosphate, 5 mM K^+ glutamate, 2 mM K^+ malate, 3 3 mM $Mg(OAc)_2$, 0 3 mM dithiothreitol, 1 0 mg/ml bovine serum albumin and 0 5 (+) or 0 1 (−) mg/ml of the rat heart mitochondrial protein In respiring mitochondria the creatine kinase rates were calculated from oxygen consumption rates [7] In mitochondria in which respiration was completely inhibited by pretreatment with 10 μM rotenone and 5 μg/mg oligomycin, the creatine kinase reaction rates were determined spectrophotometrically, using a phospho*enol*pyruvate (2 mM)-pyruvate kinase (2 IU/ml) system to regenerate exogenous ATP [7] Simulation parameters as in the Model section, except that in B $V1_{max} = 1 11$ μmol/min per mg of protein by the best approximation to experimental data $P(T A)_{in} = 0 90$

the probability of ATP binding to translocase in mitochondrial matrix $P(T A)_{in}$ is unknown and was found by a method of fitting of calculated curves to experimental data

Fig 3A shows the calculated dependences of the creatine controlled respiration rate in heart mitochondria on the ATP concentration at 25 mM creatine for the probabilities of ATP binding from matrix to translocase ranging from 0 2 to 1 0 For the given conditions, the best fitting of experimental and theoretical data was found for a probability equal to 0 835 At lower values of the probability factor the reaction rates are low and increase correspondingly with elevation of the value of this factor up to 1 0 This behavior is characteristic for the coupled system Alternatively, ADP for stimulation of oxidative phosphorylation may be produced in the creatine kinase reaction after being released into the medium – this is a case of uncoupled system (version Z, Fig 2) To estimate the contribution of the uncoupled creatine kinase system, the experiments with liver mitochondria were performed (Fig 3B) Liver mitochondria do not contain creatine kinase This enzyme was extracted in separate experiments from rat heart mitochondria and in the soluble form added to liver mitochondria In the presence of creatine the respiration rate in liver mitochondria was also dependent on the ATP concentration in the medium, however, the dependence in this case was much slower than in the case of rat heart mitochondria which contained tightly coupled mitochondrial creatine kinase (versions X and Y in Fig 2) The difference between the two curves in Fig 3B gives the minimal evaluation of the contribution of this two locally-activated complexes X and Y (Fig 2) In reality the stimulation of respiration by these locally activated complexes is significantly higher, since in experiments with isolated heart mitochondria the concentrations of creatine kinase-delivered ADP in the medium are so low that may be excluded from consideration [10]

In cooperation with Dr William Jacobus we systematically analyzed in 1982 the mitochondrial creatine kinase reaction in the forward direction both in the absence of mitochondrial respiration and under conditions of the coupling of this reaction to mitochondrial oxidative phosphorylation [7] These data are especially well-suited for mathematical modeling, since they give the quantitative description of the system in wide range of the regimens of functioning The experimental results described in that work were used in the present study as a basis for mathematical modeling Both experimental and theoretical data are shown in Fig 4 in double-reciprocal plots corresponding to reaction conditions in the presence (+) and absence (−) of oxidative phosphorylation These data were simulated by Eqn 24 at $P(T A)_{in} = 0 9$ (+) and by Eqn 7 (−) The corresponding simulation data are presented by thick interrupted (+) and thin continuous (−) lines Fig 4A, corresponding to Fig 2A in the paper by Jacobus and Saks [7] indicates the dependences of the forward creatine kinase reaction rates on the concentrations of creatine at fixed initial concentrations of MgATP MgATP concentrations are indicated at the right side of Fig 4A Fig 4B, corresponding to original Fig 2B [7], indicates the reverse situation, the dependences of the creatine kinase reaction rates on the concentrations of MgATP at fixed initial concentrations of creatine Creatine concentrations are indicated at the right of the Fig 4B

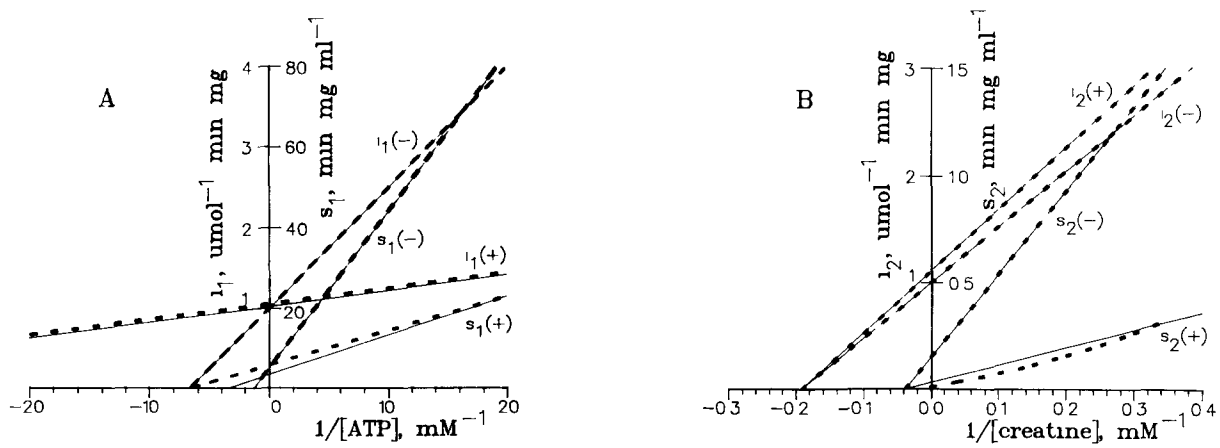

Fig 5 Secondary intercept (i) and slope (s) plots of the forward creatine kinase reaction The family of slopes and ordinate intercepts, obtained from primary double-reciprocal plots such as in Fig 4, are plotted as a function of the second substrate (A) Data from primary plots such as in Fig 4A are presented (B) Data from primary plots such as in Fig 4B are shown As the activities are varied from different batches of mitochondrial preparations, the thin lines, representing experimental secondary data, are drawn by final parameters, shown in Table I of Jacobus and Saks [7] Without (−) oxidative phosphorylation $K_{ia} = 0 75$ mM, $K_a = 0 15$ mM, $K_{icr} = 26$ mM, $K_{cr} = 5 2$ mM, $V1_{max} = 1 0$ μmol/min per mg of protein In respiring (+) mitochondria these data are 0 29 mM, 0 014 mM, 26 mM, 5 2 mM and 1 0 μmol/min per mg of protein, respectively The simulation data, presented by thick interrupted lines, are obtained from intercepts and slopes of simulated primary Fig 4 plots Parameters of simulation are as in Fig 4, $V1_{max}$ always being 1 0 μmol/min per mg of protein

For further analyses of the fitting of the theoretical data we also reconstructed the secondary kinetic plots, the dependences of the intercepts and slopes on the Fig 4A on the MgATP concentration These data are shown in Fig 5A This figure compares the reconstructed secondary plots with experimental data A similar procedure for Fig 4B is shown in Fig 5B In the absence of oxidative phosphorylation the lines were fitted completely because the same experimentally found constants were used for construction of theoretical curves It is most interesting that intercept-plot data in the presence of oxidative phosphorylation also fit completely It shows that the modeling of local activation of creatine kinase reaction at high saturating concentration of one substrate, MgATP on Fig 5A and creatine on Fig 5B, correctly describes the phenomenon In these secondary plots, the intercept plot lines intersect with abscissa axis at the points equal to $1/K_a$ or $1/K_{cr}$, respectively, the dissociation constant for MgATP and creatine from ternary enzyme-substrate complexes It is most interesting and important that the theoretical data confirm that in the case of mitochondrial creatine kinase reaction coupled with oxidative phosphorylation the value of K_m for MgATP is decreased about 10-times (Fig 5A) That shows an apparent elevation of affinity of the system for MgATP An apparent affinity of the complex of creatine kinase with MgATP to creatine is not changed and the value of the dissociation constant of creatine, K_{cr}, is 5 2 mM, both in the presence and absence of oxidative phosphorylation (Fig 5B) These results completely confirm the experimentally observed changes in kinetic constants [7–10]

In Fig 5A experimental and simulated secondary slope data in the presence of oxidative phosphorylation are close but not coinciding These slope plot lines intercepted with abscissa axis at $1/K_{ia}$, the dissociation constant for ATP from the complex CK ATP The calculated values of this constant in the presence of oxidative phosphorylation is lower (0 15 mM) than the value of experimental constant (0 29 mM) The model describes the tendency of lowering of this constant by oxidative phosphorylation [7] and confirms that the alteration of this constant by oxidative phosphorylation is much less than decrease in K_a under these conditions

System with three substrates ATP, creatine and phosphocreatine

The forward creatine kinase reaction is rapidly suppressed by end product phosphocreatine due to formation of the binary complex with PCr and ternary 'dead-end' complex with PCr and ATP (see Fig 1) Jacobus and Saks [7] studied the effect of phosphocreatine on the forward creatine kinase reaction in the presence and in the absence of phosphorylation For that the

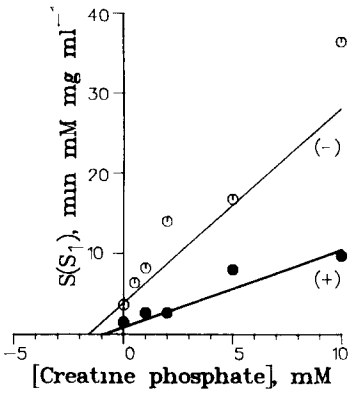

Fig 6 The secondary analysis of the slope data from competitive inhibition of creatine kinase by creatine phosphate at varying concentrations of creatine and ATP The filled and open circles represent secondary slope (S(S1)) experimental data of Jacobus and Saks [7], obtained in the presence (+) and absence (−) of oxidative phosphorylation The lines are drawn from modeling The procedure for obtaining these plots, designated for the determination of K_{icp} as the abscissa intercept, was carefully described by Jacobus and Saks [7]

reaction rate was studied in the dependence of creatine and ATP concentration at different fixed phosphocreatine concentrations The slope data from the obtained 10 families of primary double-reciprocal plots were plotted versus MgATP concentrations as in Fig 5A, each employed PCr concentration producing its own slope (S1) line Finally, the slopes from this secondary graphics (secondary, S(S1), slopes), were plotted versus direct PCr concentrations to obtain the ternary graph, with the K_{icp} value on the abcissa intercept

In Fig 6 the filled and open circles represent the final experimental secondary slope (S(S1)) data obtained in the presence (+) and absence (−) of oxidative phosphorylation, respectively They were taken from original Fig 6 of the Jacobus and Saks paper [7] The lines are drawn from modeling, we have simulated the data of all 10 primary double-reciprocal plots to obtain the primary and secondary slope data In the absence of the oxidative phosphorylation K_{icp}, the dissociation constant of PCr from the enzyme-substrate complex CK PCr, is 1 6 mM from modeling This value is equal to the experimental one, $1\,6 \pm 0\,2$ mM [7] In the presence of oxidative phosphorylation this constant value is decreased to $1\,4 \pm 0\,2$ mM in experiment and to 0 91 mM according to modeling (see Fig 6)

In the same simulation, plotting the slopes of primary intercept plots (S(i1)) vs direct PCr concentrations, we have obtained on the abscissa intercept the value of K_{Icp}, the dissociation constant of PCr from dead-end CK ATP PCr complex From modeling it is 24 mM both in the presence and absence of oxidative phosphorylation The experimental data are uncertain (K_{Icp} values were ranged from 20 to 50 mM both in

the presence and absence of oxidative phosphorylation [7], although from the another plot this value was reported to be 24 mM, p 176 of Ref 7)

Discussion

In this work we describe for the first time the quantitative model of the mitochondrial creatine kinase reaction coupled to oxidative phosphorylation system in heart mitochondria This model is based on the probability approach and in addition to the conventional kinetic equations includes the description of the ATP transfer from matrix by the adenine nucleotide translocase and its direct channeling to the active site of mitochondrial creatine kinase which is located on the other side of the inner membrane

The central problems of this approach are several assumptions concerning the values of probability factors We have assumed that the probability of the binding of ATP translocated across the membrane by translocase is equal to 1 That means that we have connected our model strictly to the concept of direct channeling of adenine nucleotides between creatine kinase and translocase This is clearly different from the concept of dynamic compartmentation of adenine nucleotides in the intermembrane space due to the proposed impermeability of the outer membrane for adenine nucleotides [25] Our proposition of the direct channeling is based on the experimental kinetic, thermodynamic and radioisotopic data [3–12] showing that the phenomenon of the functional coupling between creatine kinase reaction and oxidative phosphorylation is perfectly preserved in mitoplasts with destroyed outer membrane [9] and is lost in mitochondria in which the outer membrane is intact but the creatine kinase is released into the intermembrane space by KCl treatment [26] Also, it has been shown by immunochemical methods for mitoplasts produced from heart mitochondria that CK_{mit} and translocase are structurally closely related to each other [14] Further, this concept is also directly related to the concept developed by Wallimann et al [5,27–30], according to which mitochondrial creatine kinase forms octamers which probably are bound to the tetramers of adenine nucleotide translocator and form one multienzyme complex, translocase-CK_{mit}-outer membrane pores In this structure, ATP molecules transferred by translocase are inevitably directed to the active sites in the inner 'channels' of the octamers of CK_{mit}, and, respectively, ADP also has a decreased diffusion distance from CK_{mit} to translocase Thus, there are rather strong and good functional and structural evidence for high value of the probability of direct transfer of the ATP molecules between CK_{mit} and translocase

The most important result of this modeling is a predicted apparent decrease of the value of the dissociation constant for ATP from the ternary enzyme-substrate complex E ATP Cr, which is completely consistent with the experimental observations (see Figs 4–6) This apparent decrease has been taken to show the recycling of adenine nucleotides in the tightly-coupled system CK_{mit}-translocase-oxidative phosphorylation, an amplification effect, resulting in multiple use of small numbers of ADP on ATP and playing an important role in enhancing the regulatory signal in cardiac cells in vivo [3,4,7–12,31,32]

In conclusion, the model described in this work may be useful as a part of a more general mathematical model of phosphocreatine circuit [4,5] which should also include facilitated diffusion of the high-energy phosphate bond in the cytoplasmic equilibrium creatine kinase system [22] and coupled reaction in the myofibrils and at the cellular membranes In the complete cellular system the interaction of creatine kinase with myokinase [33] will be included However, as stated by Zeleznikar et al [33], 'adenylate kinase-catalyzed β-phosphoryl transfer may not be linked to the transfer of high-energy phosphoryls deriving from mitochondrial oxidative phosphorylation' Therefore, the adenylate kinase system will be considered in connection to glycolysis [33] Such a model will be described in our further publications

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