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Kinetic mechanisms of mitochondrial carriers catalysing exchange reactions

F. E. SLUSE, A. EVENS, C. DUYCKAERTS and
C. M. SLUSE-GOFFART
Laboratory of Bioenergetics, University of Liĕge, Place du 20-Août 7, 4000 Liĕge, Belgium

Abstract. The single-binding site or ping-pong mechanism is widely accepted for exchange reactions, catalysed by mitochondrial carriers.

However, when the most relevant approach to discriminate between mechanisms, *i.e*., kinetic study is used, the ping-pong mechanism is eliminated in favour of the sequential or ternary complex mechanism implying two binding sites simultaneously accessible to both internal and external substrates. This is the case for the oxoglutarate carrier, the aspartate/glutamate carrier and there are very strong presumptive evidences for the adenylic carrier.

Keywords. Mitochondrial carriers; kinetics; ping-pong mechanism.

Introduction

Elucidation of the whole molecular mechanism from structure/function relationship is far from being solved for any biomembrane carrier. As available structural informations cannot be interpreted unequivocally in terms of mechanism of action the most powerful approach to discriminate between mechanisms remains kinetic study. Mechanistic informations obtained from kinetics define the precise limits in which interpretations of structural and molecular data must be restricted.

Translocations by exchange through membranes must obey one of the two general types of kinetic mechanisms for a two-substrate reaction: the ping-pong mechanism or the ternary complex (sequential) mechanism.

If translocation follows a ping-pong mechanism the carrier possesses a single binding site for its substrates and it exists in two forms, one that can be loaded by the internal substrate and the other that can be loaded by external substrate. Since the transformation of one form into the other can only occur if the carrier is loaded with one substrate, the carrier performs an obligatory one-to-one exchange by binding alternately both its substrates without formation of a ternary complex S_{ext} - carrier-S_{int}. If the translocation follows a sequential mechanism the carrier possesses two binding sites, one accessible to the internal substrate and the other accessible to the external substrate and the two substrates must bind to the translocator, forming the active ternary complex, before the exchange occurs. It appears that discrimination between the two types of mechanisms gives at least direct informations on the minimum number of sites simultaneously accessible to substrates.

An initial-rate analysis permits to distinguish between the two types of mechanisms: in Lineweaver-Burk plots, a ping-pong mechanism leads to a pattern

Abbreviation used: CAT, Carboxyatractylate.

of parallel straight-lines, each corresponding to a fixed concentration of the second substrate while a ternary complex mechanism exhibits straight-lines having a common point of intercept somewhere to the left of the origin. The conclusions coming from such an analysis must be warranted by the measurements of actual initial rates (V_O) and by the knowledge of the true free-substrate concentrations to which the binding sites are exposed, at least on one side of the membrane. Moreover, the exchange under study must be catalysed by only one translocator species so that the rate is not the sum of different translocation activities and only one exchangeable substrate must be present on each side of the membrane.

Kinetic studies of mitochondrial carriers, *in situ,* have been underrated because of the technical problems that make difficult the initial-rate measurements at various concentrations of both internal and external exchangeable substrates.

An ideal situation was encountered with the oxoglutarate translocator in rat-heart mitochondria: all conditions to measure and use the initial rates properly are fulfilled and have allowed not only to eliminate the ping-pong mechanism but also to infer that the mechanism is rapid-equilibrium random and that the internal sites and the external sites are independent as shown by the convergence on the abscissa axis in double reciprocal plots. Moreover, extensive kinetic data with external oxoglutarate and malate show that the oxoglutarate translocator has an oligomeric structure, that several conformational changes occur during its activity and that the behaviour of the translocator is intrinsically asymmetrical regarding both sides of the membrane and both exchanged substrates (Sluse-Goffart and Sluse, 1986).

Initial-rate study has also allowed to eliminate the ping-pong mechanism for the aspartate-glutamate carrier (Dierks *et al*., 1988).

The adenylic carrier has been intensively studied through the molecular approach by the group of Klingenberg in Germany and the group of Vignais in France, leading to an intriguing situation where no proper kinetic data were available. The occurence of high-affinity inhibitors binding selectively either from the inside (bongkrekate) or from the outside (atractylate) has permitted an early proposal, 18 years ago, on the transport mechanism at a so-called molecular level: it was the single-binding-center gated pore mechanism with two conformational states of the carrier, the cytosol-state and the matricial-state, and this corresponds to a ping-pong mechanism. This has been claimed to be the most widespread, mechanism through which carriers translocate solutes across membranes (Klingenberg, 1989).

Methods

As isolated mitochondria contain both ADP and ATP, the first challenge to take up was to vary to a large extent the internal concentration of a single adenine nucleotide. It was reached by a depletion with pyrophosphate followed by ADP-Mg reloading procedure that leads to a wide range of ADP-concentrations (from 0 to 2·5 mM). Then, the homologuous exchange [^{14}C] ADP_{out}/ADP_{in} could be studied. The efficiency of the inhibitors was checked using different concentrations of carboxyatractylate (CAT) between 10 and 500 μM . If 10 μM CAT is large enough to block completely the exchange it does not act instantaneously. Indeed the [^{14}C] ADP content of the mitochondrial pellet is higher if CAT is added simultaneously

with ADP than if CAT is added 5 s before ADP. At 100 μM CAT there is no more difference showing that at this concentration the inhibitor acts as fast as possible.

Results and discussion

The time courses of [^{14}C] ADP accumulation in mitochondrial pellet seem to be linear between 0·3 and 1 s. The [^{14}C] uptake extrapolated to $t = 0$ increases with internal ADP concentrations for a given external [^{14}C] ADP concentration. This is due to a burst at the early periods of the progress curve as shown by fast kinetic experiments (from 35 ms) using a quench flow apparatus. The time course shows an astonishing complexity:a 10-ms lag followed by a burst lasting about 200 ms then by a quasi-linear phase (till 1 or 2 s) and lastly by a rate-decreasing phase.

The amplitude and the duration of the burst increase when external [^{14}C] ADP concentration increases at a given internal ADP concentration and increase with internal ADP concentration at a given external [^{14}C] ADP concentration. If the concentrations are low inside (< 0·2 mM) and outside (< 2 μM) the burst disappears and is replaced by a long duration lag. It must be noticed that if a rapid-equilibrium ping-pong mechanism can exhibit a burst due to the reorientation of sites induced by the addition of the external substrate such a burst would have to increase when the internal substrate content decreases contrary to our observation.

The derivative of the time course giving the uptake rate as a function of time (figure 1) clearly shows a transient phase in which the rate varies very rapidly followed by a more stable phase. As a first approximation, we have considered that the rates between 0·3 and 1 s are initial steady-state rates. Double reciprocal plots exhibit linear relationships and the straight-lines obtained for the 8 different mitochondrial contents have a common intersection point below the abscissa (0·77 μM^{-1}, 0·012 μmol^{-1} $s.\mu$ mito) suggesting that the translocator follows a ternary complex mechanism.

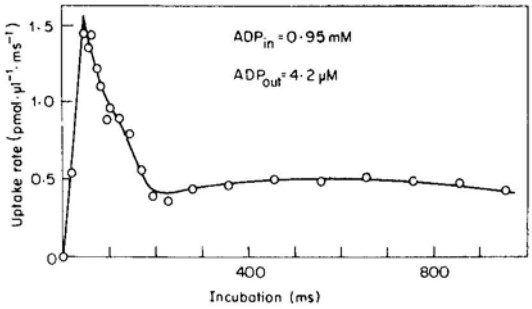

Figure 1. Time-derivative of the progress curve.

In conclusion, among the 3 mitochondrial carriers that have been studied seriously by the kinetic approach two have been proved to follow a ternary complex mechanism, oxoglutarate (Sluse-Goffart and Sluse, 1986) and aspartate-glutamate carriers (Dierks *et al.*, 1988) and we have very strong presumptive evidence for the adenylic carrier. It seems clear that a fresh look at carrier mechanisms needs to be taken.

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