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Biochimica et Biophysica Acta 1757 (2006) 1229–1236
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.elsevier.com/locate/bbabio

Transport catalysis

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Received 13 February 2006; received in revised form 6 April 2006; accepted 7 April 2006
Available online 19 April 2006

Abstract

Carrier linked solute transport through biomembranes is analysed with the viewpoint of catalysis. Different from enzymes, in carriers the unchanged substrate induces optimum fit in the transition state. The enhanced intrinsic binding energy pays for the energy required of the global conformation changes, thus decreasing the activation energy barrier. This "induced transition fit" (ITF) explains several phenomena of carrier transport, e.g., high or low affinity substrate requirements for unidirectional versus exchange, external energy requirement for "low affinity" transport, the existence of side specific inhibitors to ground states of the carrier, the requirement of external energy in active transport to supplement catalytic energy in addition to generate electrochemical gradients.

Keywords: Transport; Catalytic energy; Induced fit; Active transport; Electrochemical gradient

Whereas the concept of catalysis is well conceived and widely exploited in enzymes [1,2], it is infrequently applied with its ramifications to transport [1,3–7]. For illustrating the crucial elements of carrier mediated catalysis a comparison to familiar enzymatic catalysis is useful [4,8,9]. Reaction theory requires that the catalyst, e.g., the enzyme, assist in transforming the substrate into a transition state. The binding site is constructed not to bind optimally the substrate but to fit the distorted substrate in the transition state. Thus by using energy generated from the optimum binding in the transition state, the substrate is readied for the subsequent and spontaneous chemical alteration. In contrast, carriers catalyse transport of substrates through membranes without chemical changes and therefore the concept of catalysis has received little attention in the transport field. In particular, it was not clear what a transition state as an essential ingredient of catalysis would mean and look like. Pertinent models of carriers were concerned with the global changes comprising the binding site reorientation between the "out" and "in"-side configurations. But the question was barely addressed, what happens in between these two "ground states".

The single binding center gated pore model (SBGP) has been the underlying mechanism for the present analysis of transport catalysis [10–13]. It provides a good example for simple single substrate enzyme catalysis. The SBGP was first established on the molecular level with the AAC using specific inhibitor ligands. With two different, side specific inhibitors the carrier population could be entirely transferred from the out- to the in-side ground state and vice-versa. The presence of ADP or ATP was obligatory as catalysts of this transition coupled to the translocation of one substrate molecule. The SBGP is manifest in a wide range of transporters, encompassing unidirectional facilitators, exchange type transporters, and primary or secondary active transporters. The emergent structures of carriers of widely different provenance provide a striking support to the SBGP model and discard tandem and multiple binding site models [14–20].

Based on extensive observations in the mitochondrial membrane, in the isolated state, and in the reconstituted system [21,22], the intricacies of the transition between the "c" (out) and "m"(in) states of the mitochondrial ADP/ATP carrier (AAC), with often seemingly contradictory results, challenged us to comprehend the underlying mechanism of transport catalysis. In line with reaction theory and in analogy to enzymatic catalysis, we introduced the transition state, where the carrier–substrate complex assumes an intermediate configuration between the two ground states [4,8]. Here the substrate induces an optimum fit and thus generates a maximum intrinsic binding energy which drives the carrier substrate complex into and through the transition state between the ground states.

With the advent of crystal structures of some carriers, molecular modelling of the transport mechanism and the forces

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doi:10.1016/j.bbabio.2006.04.010

involved become more precise and challenging. But in recent models based on 3D structures, intermediate steps between the in and out states were not considered or only marginally described [15,23,24]. Here we want to demonstrate that the concept of the induced transition fit (ITF) may provide an important guide to interpret the substrate–protein interactions involved in the translocation process.

1. Enzyme and carrier catalysis cycles

A comparison of the simple catalytic cycles of enzyme and carriers illustrate similarities and differences between these two protein mediated types of catalysis (Fig. 1). Whereas after product release the enzyme is recuperated in a state ready for the next catalytic cycle, the carrier transformed after release of the substrate and the catalytic cycle is complete only after the second "return" branch. In balance, the carrier catalyses a vectorial reaction of the substrate, by facilitating the translocation through a diffusion barrier. In some way, the roles of the protein and the substrate are reversed: the substrate remains chemically unchanged but the protein has assumed a different configuration with opposite access paths to the binding center. One may regard the substrate rather than the carrier as the catalyst.

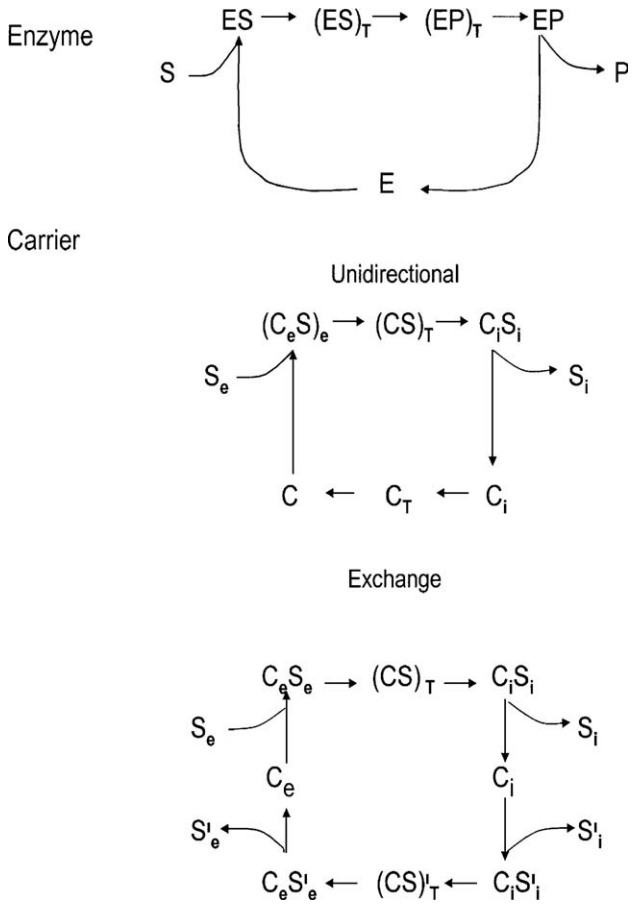

Fig. 1. Catalytic cycles for a simple enzyme reaction and for unidirectional (uniport) and exchanging (antiport) carrier transport. During the cycle, the carrier assumes two different ground states, with the binding site directed outside or inside. As a result, the full carrier cycle has two transformation branches. At an intermediate stage, the carrier enters the transition state.

Whereas in the analysis of enzyme mechanism, the transition state is well conceived, it is not obvious to introduce this pivotal step to transport. Indeed, the need for introducing the transition state becomes more rational only by considering the often important changes in the carrier structure during the translocation. Evidently, these global structural shifts require intermediate steps, some of them with the highest energy level qualifying as classical transition states. Most importantly, this catalysis is highly selective for the substrate structure, often surpassing that of enzymes dealing with the same substrate.

In enzymes in the transition state, the interaction between a distorted substrate and an adapted binding site is optimised [25]. However, the conformation changes are limited and focussed on the catalytic centre. In carriers, the substrate is unchanged but the protein undergoes a global conformation change in the transition state, midway in the transformation between the in- and out-states. How is the energy for these important changes generated? Similar as in enzymes, the substrate is not optimally bound in the ground states but, different from enzymes, the unchanged substrate induces a major reorganisation in the binding centre going into the transition state to attain an optimum fit (Fig. 2). In the concomitant global structure change, the gates are partially opened and closed respectively. This substrate induced optimum fit of the binding site leading to the transition state (induced transition fit, ITF) is the essence of the carrier catalysis.

An energy reaction profile illustrates the implications of the ITF (Fig. 3). At first, substrate binding releases little energy because of poor fit to the ground state. On proceeding into the transition state, the increased substrate protein interaction forces produce a larger "intrinsic" binding energy, The energy costs of the concomitant reconfigurations of the carrier, shown by the high energy barrier in the substrate less transition state (C_T), are paid for by the intrinsic binding energy such that in balance the energy barrier for the carrier substrate complex in the transition state is low. The energy difference of the carrier transition states without and with substrate corresponds to "catalytic energy" which is responsible for the substrate linked rate acceleration. In other words the catalytic energy necessary for transport is recruited from the substrate–protein interaction forces reaching a maximum in the transition state. Also in enzymes substrates can induce changes in the active center, which may even propagate to some other "background" residues along hydrogen and ionic bond relays [25]. But those changes are generally small and centred, whereas in substrate induced transition fit (ITF) of carriers the changes are large and global, focused not only on the binding center but also on the gating region. Relays of interacting residues emanate from the binding center towards the gating regions along the translocation path as illustrated in Fig. 2. Hereby, intermediate states may be partially stabilised as shown in the energy reaction profile of Fig. 3. Although extensive regions of the protein are modified, these changes follow a precise trajectory in a multidimensional energy space, coordinating all residues involved. Changes may involve translational and rotational movements, bending and tilting of transmembrane helices,