Regulation of Fatty Acid Oxidation


Received 9 December 1993

Metabolic control analysis of hepatic β-oxidation: the top-down approach
Patti A. Quan* and Ron A. Makins
Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, U.K.

Hepatic β-oxidation
For β-oxidation to occur in the mitochondrial matrix of liver cells, fatty acids, the pathway substrates, must cross the inner mitochondrial membrane. Long-chain fatty acids are transported across the membrane via a three-step system involving carnitine palmitoyltransferase (CPT) I, the carnitine carrier and CPT II. CPT I converts acyl-CoAs to external acyl-carnitines so that they can be imported on the carnitine carrier. CPT II reconverts the imported acyl-carnitines to acyl-CoAs which then undergo β-oxidation. Medium- and short-chain fatty acids enter the mitochondrial matrix independently of this mechanism. Reducing equivalents produced by β-oxidation, NADH and FADH2, feed electrons into the electron-transport chain (ETC) as substrates of oxidative phosphorylation, and acetyl-CoA is oxidized to CO2 in the Krebs cycle or directed to ketone body synthesis in the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) cycle of ketogenesis. So, the mitochondrial β-oxidation pathway for long-chain fatty acids can be viewed as two blocks of reactions: one external to the matrix, producing external acyl-carnitines (CPT I) and the other consuming them (carnitine carrier, CPT II, β-oxidation, Krebs cycle, HMG-CoA cycle, ETC and ATP synthase). The

Abbreviations used: CPT, carnitine palmitoyltransferase; ETC, electron-transport chain; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; TDCA, top-down control analysis; TDEA, top-down elasticity analysis; TDRA, top-down regulation analysis.
*To whom correspondence should be addressed.
two blocks are connected by a common intermediate, external acyl-carnitine.

**What are the top-down approaches to metabolic analysis?**

To measure the distribution of control in the mitochondrial β-oxidation pathway, we have applied top-down control analysis (TDCA) [1-3], an experimental method based on metabolic control analysis (MCA) devised by Kacser and Burns [4] and by Heinrich and Rapoport [5]. Our top-down analyses build on an earlier bottom-up analysis (see below) of a truncated system by Kunz [6], which prompted our research. Although his simple system excluded CPT I, the reputed 'rate-limiting' enzyme of long-chain fatty acid oxidation and ketogenesis, the results of Kunz's analysis demonstrated clearly that control is distributed over the pathway and that the distribution changes with changes in conditions. His findings and ours suggest that the popular concept of a single rate-limiting enzyme of β-oxidation is not adequate.

MCA was designed to investigate control exerted by single intermediates, steps or enzymes. By repeated application and subsequent summing of the contributions of all the individual components of the system, a full description of the control structure of the whole pathway can be obtained from this method, which we call the 'bottom-up' approach. In contrast, our approach is known as the 'top-down' approach because we analyse the distribution of control over the whole pathway, by one or two simple experimental manipulations, thus gaining an immediate overview. Repeated application of this type of analysis yields information about the individual steps involved. Both approaches, therefore, are different means to the same end and ultimately give the same information. To apply top-down control analysis, however, it is not necessary to use specific inhibitors or a means to manipulate levels of enzymes genetically, and if one is interested in control structure of the whole pathway, rather than control exerted by one particular step, it is clearly the more appropriate technique.

For clarity, in this paper, we have chosen to describe in detail top-down analyses of a very simple system, which is shown in Figure 1. We do however discuss briefly our conclusions drawn from similar studies of more 'physiological' and complex systems.

We now believe that control analyses alone only ever reveal part of the picture: they identify control sites that are most likely to be sensitive to regulation by effectors. Therefore, to identify the sites most sensitive to effectors of β-oxidation, the targets of effector action, we are performing top-

---

**Figure 1**

The system used for the top-down analyses described in the text

Isolated rat liver mitochondria (2 mg ml⁻¹) were incubated with 20 μM palmitoyl-CoA at 30°C for 2 min in a medium containing 120 mM KCl; 5 mM Hepes; 1 mM EGTA; 5 mM potassium phosphate; 1 mM ATP; 1 μg mg⁻¹ oligomycin; 1.6 μM carbonyl cyanide p-trifluoromethoxyphenylhydrazone; 10 mM malonate; 20 μM fluorocitrate; 10 μM rotenone and 17.5 mM [¹H]carnitine (0.35 μ Ci μmol⁻¹, pH 7.2, 30°C). Ketone body mixtures, β-hydroxybutyrate:acetoacetate, were added at a ratio of 10:1, 7.5:2.5, 5:5, 2.5:7.5 and 1:10 at a constant total concentration of 10 mM to manipulate the flux. Malonyl-CoA was added at 0, 5, 10, 20 and 30 μM for each titration.
down elasticity analyses (TDEAs). These experiments employ a further extension [7] of MCA.

Finally, to gain an even fuller picture, an overview of how the pathway is regulated, i.e. how regulation is exerted via effectors acting on sensitive control sites, we are applying top-down regulation analyses (TDRAs). These interpret and combine information from both control and elasticity analyses.

What do we measure?

Flux control coefficients

For application of either approach to MCA, we change a parameter which we can set from outside the system, e.g. the concentration of an enzyme, and measure the resulting change in a variable, e.g. the flux through the pathway, when everything in the defined system has been allowed to settle to a new steady state. From these measurements, we can calculate flux control coefficients (C). These are quantitative measures of the control of a variable (steady-state rate or [intermediate]) by a particular parameter (enzyme or step). For the bottom-up approach, the flux control coefficients would describe the control exerted by individual steps in the pathway. For the top-down approach, however, the coefficients are group coefficients and refer to whole sections, or blocks, of the pathway under consideration. This is reflected by the use of a star (C'). For example, in our analyses, the flux control coefficients C_{\beta-ox} or C_{\beta-ox}' describe the control exerted by the blocks of enzymes involved in external palmitoylcarnitine production and \beta-oxidation (subscripts CPTI or \beta-ox) over the pathway flux (superscript \beta-ox). They express by how much flux through \beta-oxidation changes (the fractional change) in response to an infinitesimal fractional change in the effector with everything else in the system held constant. Elasticities are properties of the step under consideration and are not system properties. They are however related to flux control coefficients, and therefore the system, via the connectivity theorem or property. In our simplified system, the amount of control exerted by the two blocks of reactions depends on the kinetic responses, i.e. the group elasticity coefficients, of the two blocks to the intermediate, external palmitoylcarnitine. The connectivity theorem states that the sum of the product of the flux control coefficients and a set of elasticities to a particular pathway effector, e.g. the intermediate, is zero. This relationship means that if we can measure the elasticities of the two blocks in our system to their common intermediate, we can use the summation and connectivity theorems to calculate the flux control coefficients and, therefore, the response coefficients describing our system.

Response coefficients

Partial response coefficients describe how much an infinitesimal fractional change in an effector (which could be the common intermediate, any other internal or an external effector) changes the pathway flux via effects on a particular block. They are calculated from the product of the relevant elasticity and flux control coefficient. So, in our system, the partial response coefficients C_{\beta-ox}R_{\beta-ox}' and \beta-oxR_{\beta-ox}', describe how sensitive each of the two blocks of reactions are to an effector, X, where X could be palmitoylcarnitine or malonyl-CoA. Therefore, they tell us how effectors regulate whole sections of the pathway by action on sensitive control sites. The sum of the partial response coefficients equals the group coefficient (*R). Therefore, this can be calculated or measured directly as the fractional change in pathway flux as a result of an infinitesimal fractional change in the effector with everything else in the system constant. In our system, C_{\beta-ox}R_{\beta-ox}' + \beta-oxR_{\beta-ox}' = *R_{\beta-ox}'. Therefore, if we can measure fluxes, and changes in the intermediate, we can calculate elasticities, flux control coefficients and, therefore, response coefficients (Figures 2 and 3).

Top-down control analysis: where does control lie?

We have carried out a series of TDCAs in isolated rat liver mitochondria, around the intermediate external palmitoylcarnitine. Our aim was to try to establish the distribution of control over \beta-oxidation between CPT I and the enzymes of \beta-oxidation under 25 different sets of conditions.
Flux control coefficients over β-oxidation at constant palmitoyl-CoA concentration (20 μM)

The two sets of flux control coefficients were calculated from the two sets of elasticities to the common intermediate, palmitoylcarnitine, using the summation and the connectivity theorems [1,2]. The elasticities were taken directly from plots of external [14C]-palmitoylcarnitine (% change) against β-oxidation, measured as O₂ consumption (% of control).

To analyse the system, we carried out two separate manipulations. Firstly, we manipulated the kinetics of CPT I by titrating through five different malonyl-CoA concentrations and measured the response of the enzymes of β-oxidation, by measuring changes in O₂ consumption, to the resulting changes in intermediate concentration. This allowed us to calculate the elasticities of the group of enzymes to the intermediate. The second manipulation involved titrating through five different increasing β-hydroxybutyrate:acetoacetate ratios, at each malonyl-CoA concentration, where the total β-hydroxybutyrate:acetoacetate was held constant at 10 mM. By a similar method, this allowed us to calculate the elasticities of CPT I to the intermediate. From these sets of elasticities, we were able to calculate the two sets of 25 flux control coefficients shown in Figure 2.

It is clear from Figure 2 that control over β-oxidation is shared between the two blocks of reactions although, at low fluxes, the control lies predominantly within the β-oxidation block and CPT I never exerts more than 0.5 of the control under the conditions studied. Control is shared equally at 30 μM malonyl-CoA and high flux. These results suggest that when β-oxidation is inhibited, by a high NADH:NAD ratio, the control exerted by CPT I is very low as its capacity far exceeds that of β-oxidation. In contrast, when β-oxidation is not inhibited and the CPT I is inhibited by high malonyl-CoA concentrations, then control shifts to CPT I. These analyses suggest that the consumer block of reactions contains the best site(s) at which to apply regulation by effectors.

Top-down elasticity analysis: where do effectors act?

Further useful information can be extracted from the same titrations by applying TDEAs. To demonstrate the use of TDEAs we applied such analyses to confirm the target site of malonyl-CoA. Under the conditions studied, steady-state rates are set by the kinetic responses of the two blocks of reactions to the intermediate, palmitoylcarnitine. Effectors, such as malonyl-CoA, change the steady-state rates by changing the kinetic responses of the blocks to the intermediate. Therefore, the elasticities of the two blocks to the intermediate will only change in the presence or absence of an effector if the blocks contain target site(s) sensitive to that particular effector. By comparisons of the elasticities to the effector, in the presence or absence of the effector when everything else is held constant, the target site(s) can be identified.

From the same dataset obtained from the TDCAs, we calculated the sets of elasticity coefficients of the two blocks of reactions to malonyl-CoA at four different malonyl-CoA concentrations each at four different fluxes. These results are shown in Figure 3(b).

The sets of elasticity coefficients in Figure 3(b) describe the sensitivity of each block to malonyl-CoA under the specific conditions studied. At all fluxes and malonyl-CoA concentrations, the β-oxidation block is insensitive to malonyl-CoA. But all the values for the elasticity coefficients are negative, indicating the inhibitory effect of malonyl-CoA on the whole pathway. It is clear from these anal-
Regulation of Fatty Acid Oxidation

Top-down regulation analyses

(a) Flux control coefficients of CPTI and the enzymes of β-oxidation over β-oxidation flux. (b) The elasticity coefficients were calculated from the same dataset as a fractional change in flux through each block for a fractional change in malonyl-CoA concentration. (c) The partial response coefficients were calculated as the product of the relevant flux control and elasticity coefficients. (d) The partial response coefficients were summed to give the group response coefficients. MCoA, malonyl-CoA.

Figure 3

Top-down regulation analyses

TDEAs pinpoint the target sites of effectors, but to establish how effectively and at which site(s) effectors regulate a pathway, it is necessary to apply regulation analyses. Once again, by using the same datasets, it is possible to calculate the partial and group response coefficients. These are shown in Figures 3(c) and 3(d).

Specific conclusions

TDCAs have shown us that, under most of the conditions that we have studied, control over the pathway lies mainly within the β-oxidation block. TDEAs show that, except at low malonyl-CoA concentrations, CPT I is sensitive to malonyl-CoA.

yses that malonyl-CoA exerts its effects predominantly at CPT I.

TDEAs pinpoint the target sites of effectors, but to establish how effectively and at which site(s) effectors regulate a pathway, it is necessary to apply regulation analyses. Once again, by using the same datasets, it is possible to calculate the partial and group response coefficients. These are shown in Figures 3(c) and 3(d).

The plot of the partial response coefficients, which are the products of the flux control and elasticity coefficients (Figures 3a and 3b), clearly shows that malonyl-CoA inhibits the pathway through its effect on CPT I. The sets of group response coefficients, the sum of the partial response coefficients (Figure 3d), show that at high flux and high malonyl-CoA concentrations, the pathway is inhibited via inhibition of CPT I by malonyl-CoA.
TDRAs, through the partial and group response coefficients, confirm that, under every condition that we have studied, malonyl-CoA exerts its effects over the pathway via CPT I. These conclusions apply only to our system and our conditions. Yet our TDCAs in a more physiological system, including ketone body and ATP synthesis in coupled mitochondria, show that in state 3 CPT I has most of the control and in states intermediate between 3 and 4 the control is shared. That the simple TDCAs show most of the control resides with the β-oxidation block is potentially misleading, as the TDRAs show malonyl-CoA regulates the pathway via its effects on CPT I.

General conclusions

It is clear from this study that TDCA only tells us how control over a pathway is distributed. TDEA tells us where effectors act. Only by doing a full TDRA for each effector can we reveal how effectors regulate a pathway by their actions on sensitive control sites.

We thank Dr Martin D. Brand and Dr Victor A. Zammit for helpful discussions. The research described here was supported by an Agricultural and Food Research Council Link Grant.


Received 10 January 1994

Processivity and fatty acid oxidation

Paul A. Srere* and Balazs Sumegi†

*Department of Veterans Affairs Medical Center and Biochemistry Department of The University of Texas Southwestern Medical Center at Dallas, 4500 S. Lancaster Rd., Dallas, Texas 75216, U. S. A. and †University Medical School, Department of Biochemistry, H-7624 Pecs, Szigeti ut 12, Hungary

Introduction

Metabolic pathways can be termed ‘processive’ if they progress in an orderly step-wise fashion and if the metabolic intermediates are expeditiously used by the following enzyme of the pathway. Chains of reactions proceed by small chemical steps as each enzyme conversion is a small chemical change. A pathway is considered ‘highly processive’ (tight channelling) if the intermediates are never in equilibrium with the bulk water of the cell (compartment). Examples of this are protein and nucleic acid biosynthesis, fatty acid synthesis, and tryptophan synthase. From these examples, one can deduce that processivity is not limited to one mechanism. Other pathways are ‘partially processive’ (loose channelling) in that some of the intermediates serve several metabolic functions so that they must exist in partial equilibrium with the bulk water of the cell.

The biosyntheses and degradations that one encounters in the living cell usually involve a host of intermediates. How many of these intermediates serve a cellular function other than its occurrence in its particular pathway? An examination of a number of inter-related metabolic pathways reveals that at least 70% of metabolic intermediates serve only a single function in the cell [1]. The range of $K_m$s for many enzymes (10⁻⁸ to 10⁻³ M) means that if the components of a cell were in a random array, then many molecules of each substrate would be required. For metabolites used in one process only, this arrangement would appear to require excess energy to maintain the high concentrations of metabolites and would utilize much of the solvent capacity of a cell.

Compartmentation

In order to make metabolism more ‘efficient’, living cells have developed a number of strategies. Two

Abbreviations used: CPT, carnitine palmitoyl transferase outer mitochondrial membrane; ETF, electron-transfer flavoprotein; ETF-red, ETF-ubiquinone oxidoreductase; TCA, tricarboxylic acid.

*To whom correspondence should be addressed.