10 Irreversible reactions in metabolic simulations: how reversible is irreversible?

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Mathematically and thermodynamically there are no irreversible reactions in chemistry, and all enzymes catalyse reversible reactions. All reactions have finite standard Gibbs energies, which is just another way of saying that they all have finite equilibrium constants. Nonetheless, for an enzyme like pyruvate kinase, with an equilibrium constant of the order of 10^5, one may feel that this is just a mathematical nicety and that the thermodynamic necessity for the reverse reaction to be possible has no practical consequences for cell physiology, and in practice many metabolic reactions are conventionally regarded as irreversible. This typically means that they have equilibrium constants of the order of 1000 or more, though when the mass action ratio in the cell also strongly favours the forward direction a reaction with a substantially smaller equilibrium constant than this can be regarded as irreversible. For example, the hexokinase reaction has an equilibrium constant that is less than 250 at pH 6.5 (Gregoriou *et al.*, 1981), but the reaction is still considered irreversible because in normal physiological conditions the concentration of glucose is always much greater than that of glucose 6-phosphate and the concentration of ATP is usually greater than that of ADP.

In metabolic simulations the difficulty and inconvenience of using complete reversible rate equations for all the enzymes is aggravated by the fact that for nearly all "irreversible" enzymes virtually no kinetic measurements of the rates of the reverse reactions, or even of the effects of products on the rates of the forward reactions, have ever been reported. In the absence of any data, therefore, one is forced to choose between ignoring the reverse reaction altogether or guessing what its kinetic parameters might be on the basis of the values of the equilibrium constant and of the kinetic parameters for the forward direction. It would hardly be surprising if many simulators faced with such an unappealing choice preferred the easier option of ignoring reverse reactions, apparently safe and no worse than just guessing the parameter values.

Our view is that it is always best to use reversible equations in metabolic sim-

ulations for all processes apart from exit fluxes, and similar sentiments are expressed in the documentation for the simulation program Gepasi (Mendes, 1993). Even so, we were surprised how large the effect of allowing for the reverse reaction of pyruvate kinase in a model of glycolysis in *Trypanosoma brucei* proved to be (Eisenthal & Cornish-Bowden, 1998; Cornish-Bowden & Eisenthal, 2000). With this step treated as strictly irreversible transport of pyruvate from the cell had a flux control coefficient of zero, as expected for a step following an irreversible step. What was not expected, however, was that allowing for the reverse reaction of pyruvate kinase by assuming reasonable parameter values consistent with the equilibrium constant of about 10^5 would perceptibly change all of the flux control coefficients in the system and that export of pyruvate would go from having no flux control at all to having the second largest flux control coefficient in the system.

The model of glycolysis in *Trypanosoma brucei*, developed originally by Bakker *et al.* (1997), is relatively complex, with 20 steps in four compartments; many of the steps have multiple substrates and products, and the metabolite concentrations as a whole are constrained by four different conservation relationships, one of them highly complicated and unintuitive. All of this makes it a rather unsuitable model for analysing the effect of allowing for the reversibility of pyruvate kinase, and one may suspect that the unexpected behaviour observed was related in some way to the complexity of the model. We therefore decided to investigate the question in the context of a model simple enough for the results to be fully understood.

We have therefore compared the results of simulating the metabolic behaviour of the four models illustrated in Fig. 10.1. The most complete version of this model is shown at the top-left as Fig. 10.1a. In this version there is a feedback loop from the end-product S_5 to the first step, catalysed by E_1, which follows the reversible Hill equation (Hofmeyr & Cornish-Bowden, 1997) with a Hill coefficient of 2; in addition, E_4 is represented by a reversible rate equation even though the equilibrium constant of 5×10^5 might be regarded as large enough for the reverse reaction to be neglected. In two of the variant versions of the model, 1c and 1d, the feedback effect of S_5 on E_1 is absent, and in models 1b and 1d the reaction catalysed by E_4 is made strictly irreversible.

The four versions of the model thus include all possibilities necessary for deciding whether taking proper account of feedback inhibition is more or less important than allowing for the small degree of reversibility in a reaction with a very large equilibrium constant. If allowing for this small degree of reversibility is trivial, whereas ignoring a feedback loop is crucial, we should expect the results from models 1a and 1b to resemble one another and to differ from those from models 1c and 1d. However, if it proves essential to allow for even a small degree of reversibility, but unimportant to take full account of a feedback loop, then we should expect models 1a and 1c to resemble one another and to differ from models 1b and 1d.

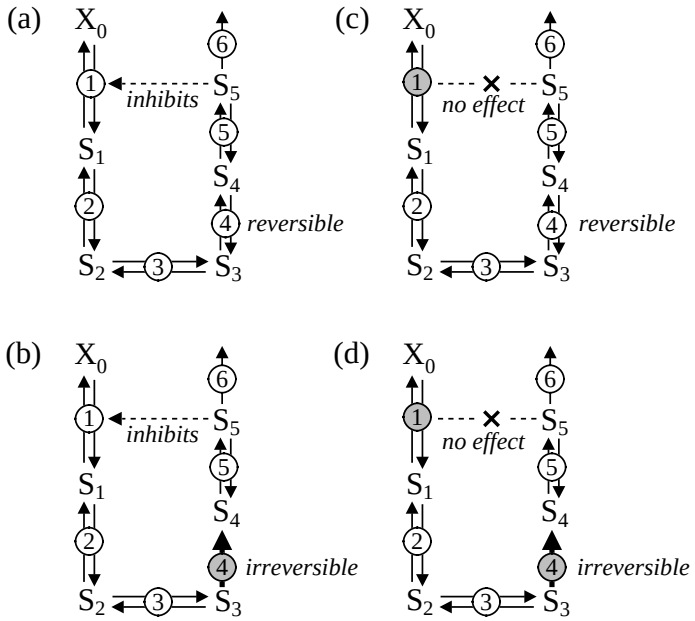

Fig. 10.1 A model pathway with (a,b) and without (c,d) feedback inhibition of the first step by the end product, and taking account (a,c) or not (b,d) of a very small degree of reversibility in the fourth step. The rate of the reaction catalysed by E_1 was $10X_0(1-S_1/25X_0)(X_0+S_1)/[1+(X_0+S_1)^2+S_5^2]$; when there was no feedback inhibition the term in S_5 was omitted from the denominator. Step 2 was treated as an equilibrium with equilibrium constant of 1. E_3 and E_5 followed reversible Michaelis–Menten kinetics with forward and reverse limiting rates of 8 and 0.5 respectively for E_3 and 12 and 2 respectively for E_5, and forward and reverse Michaelis constants of 2 and 3 respectively for E_2 and 0.5 and 2 respectively for E_5. In models (a) and (c) E_4 also followed reversible Michaelis–Menten kinetics, with forward and reverse limiting rates of 10 and 0.001 respectively, and forward and reverse Michaelis constants of 0.5 and 25 respectively; in models (b) and (d) the ordinary irreversible Michaelis–Menten equation was used with limiting rate 10 and Michaelis constant 0.5 (effectively setting the reverse limiting rate to 0 and the reverse Michaelis constant to infinity). The exit flux catalysed by E_6 followed Michaelis–Menten kinetics with Michaelis constant 0.5 and a limiting rate V_6 that was varied to simulate changes in demand. The concentration of X_0 was fixed at 10. All simulations were done with Gepasi (Mendes, 1993), version 3.21, which was downloaded from http://www.ncgr.org/software/gepasi/.

So far as the flux and the flux control coefficients are concerned it turns out that neither of these expectations is fulfilled. As illustrated in Fig. 10.2, three models give qualitatively very similar behaviour but the fourth, model 1d, is very different. In other words one can ignore either feedback loops or reversibility with relatively little effect on the flux properties, but one cannot ignore both. Although at first sight perhaps surprising, both aspects of this result are consistent with what was known before. The contribution of feedback inhibition to metabolic regulation has been previously analysed in several models without any internal irre-

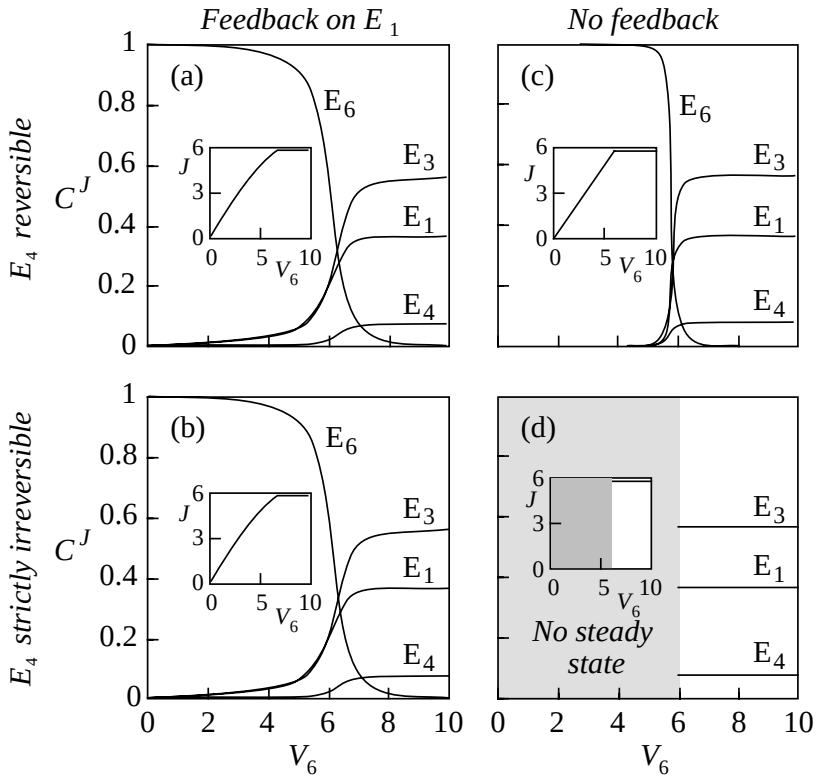

Fig. 10.2 Variation of the distribution of flux control as a function of demand for the four models illustrated in Fig. 10.1. The layout is the same as in Fig. 10.1, and the letters (a–d) refer to the same four cases. The variation of the flux J with the demand is shown in the inset to each plot.

versible steps but of varying degrees of complexity (Hofmeyr & Cornish-Bowden, 1991; Cornish-Bowden *et al.,* 1994, 1995), with consistent results: in all cases the importance of feedback inhibition (and the degree of cooperativity of such inhibition) has been found to lie not in more effective flux regulation but in allowing metabolite concentrations to remain relatively little changed when the flux changes. Similarly, the finding that a step that is isolated from the rest of the system by an irreversible step cannot exert any flux control is a classical result in control analysis.

Model 1b gives results that resemble those from the complete system much more than they resemble those from model 1d, even though it interposes a completely irreversible step between the last two enzymes and the rest of the pathway. The explanation is that even though E_5 and E_6 follow an irreversible step they are not isolated by it from the rest of the pathway because they can communicate with it through the feedback loop. So far as flux control is concerned, therefore, what is important is that the end product can communicate with the early steps in a pathway; it does not matter whether this communication is along the chain or via a feedback loop. A practical illustration is provided by serine biosynthesis

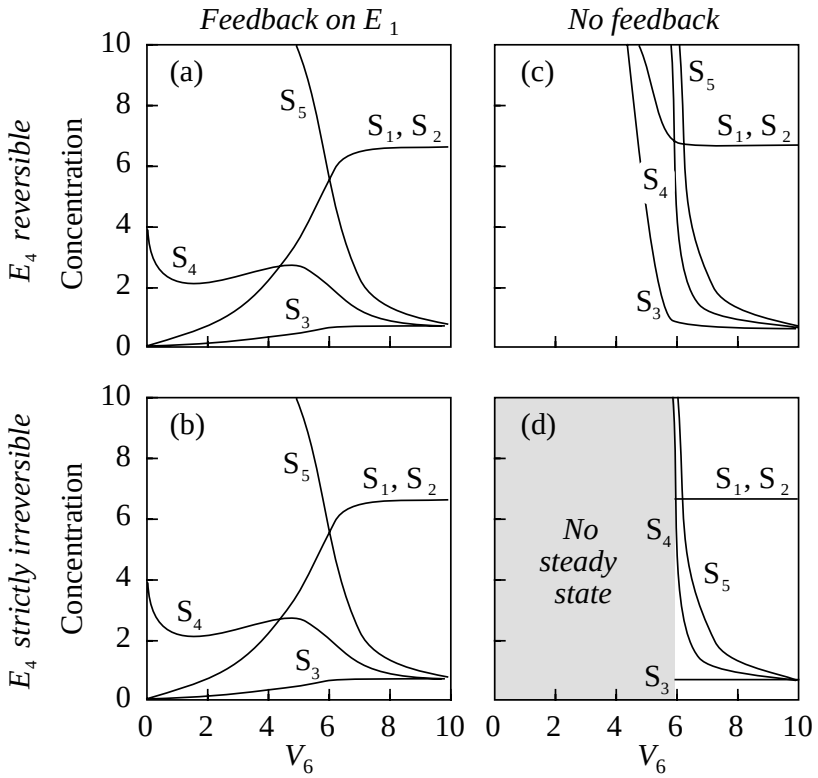

Fig. 10.3 Variation of metabolite concentrations as a function of demand for the four models illustrated in Fig. 10.1. The layout is the same as in Figs. 10.1–10.2, and the letters (a–d) refer to the same four cases.

in bacteria and mammals (Fell & Snell, 1988): in bacteria the end product serine acts as a feedback inhibitor, but in mammals it does not, acting only as an ordinary product inhibitor; in both cases, however, it regulates its own synthesis quite effectively. Moreover, in the case of an almost irreversible reaction it is not the near-zero term in the numerator of the rate expression that is essential for communication with the earlier steps but the terms in product concentration in the denominator. In other words it is not reversibility as such that is important but the possibility of product inhibition.

As in previous studies of feedback inhibition (Hofmeyr & Cornish-Bowden, 1991; Cornish-Bowden *et al.,* 1994, 1995), the crucial difference between models with and without such inhibition is not in the flux behaviour but in the changes in metabolite concentration that accompany changes in flux. In the present case, illustrated in Fig. 10.3, the concentration patterns for models with feedback inhibition are virtually identical whether or not the reversibility of the fourth reaction is allowed for (Fig. 10.3ab). The two cases without feedback inhibition (Fig. 10.3cd) are not identical but are still much more similar than the corresponding patterns for the flux (Fig. 10.2cd) might lead one to expect. In the fully irreversible case (Fig. 10.3d) lowering the demand to below the flux delivered by E_1 causes the con-

centrations of the intermediates that follow the irreversible step to rise to infinity, so that no steady state is possible; in the almost-irreversible case (Fig. 10.3c) these concentrations also rise to very high values, albeit not to infinity.

If flux regulation were the only consideration, one might consider Fig. 10.2c to represent the ideal, with flux strictly equal to demand until the limit that the system can deliver. In contrast, in Fig. 10.2ab the proportionality is only approximate and the flux is always a little less than the demand. However, this imperfection is very slight, and the "perfection" of Fig. 10.2c is bought at the very heavy price of complete loss of concentration regulation (Fig. 10.3c).

Gaining insight into the design of metabolic regulation has been only part of our objective in this investigation. We have also been concerned to determine when it is safe in a metabolic simulation to treat reactions with very large equilibrium constants as strictly irreversible. The conclusion is that no matter how large the equilibrium constant one must not allow a step to completely isolate one part of a pathway from the rest. If there is a feedback loop that allows communication around an irreversible step then the behaviour is virtually identical whether the reversibility of the nearly irreversible step is allowed for or not. Similarly, if terms for product inhibition are included in the denominator of the rate expression then it will make little or no difference whether the small negative term is included in the numerator or not. Considered in these terms the initially puzzling observation in the model of glycolysis in *Trypanosoma brucei* makes good sense and does not require any explanation in terms of obscure properties arising from the complexity of the model. In the original model with pyruvate kinase treated as strictly irreversible (Bakker *et al.*, 1997) there was no feedback loop around this enzyme, and no possibility of product inhibition was allowed for. Consequently pyruvate transport was completely isolated from the rest of the pathway and any effects that it could have had on the regulatory structure were concealed; they were only revealed when communication between pyruvate and the earlier steps was permitted.

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