BIOCHEMICAL LETTERS

Flux ratios, induced transport and tracer perturbation

Rebholz and Northrop (1993) have recently published a paper in the *Biochemical Journal* criticising a number of aspects of a paper that I published (Britton, 1973) on single-substrate single-product enzyme reactions. I am very grateful to Cornish-Bowden (1994) for his rebuttal in your journal of their criticisms that: (i) my conclusion that the non-competitive term in the steady-state rate equation disappears when the isomerization of the free enzyme is very slow is incorrect, and (ii) I did not take into account all of the reaction steps in the enzyme reaction. With respect to (ii), I gave consideration to the number of steps that could be detected by steady-state kinetics (including the use of labelled substrates) in Britton (1966b) and concluded that, for a single-substrate single-product reaction, a model with only a single enzyme–substrate complex would be homioiketic with all models containing additional intermediate steps. Thus, by suitable adjustment of the rate constants, a model with a single enzyme–substrate complex will give patterns of steady-state velocities as functions of substrate concentrations that are identical with those given by the more complex systems. Only steps that can be detected by steady-state kinetics should be included in the initial analysis. If, at a later stage, one should wish to take into account additional steps, they can be incorporated, without recalculation of the overall rate equations, by the equivalent reaction technique (Britton, 1977). In retrospect, I regret, however, that I did not point out that there is an alternative, minimal, homioiketic model in which the reaction of the enzyme with the substrates is fast and the interconversion of the two complexes is slow (Figure 1).

Rebholz and Northrop (1993) also treat certain aspects of isotopic kinetics in a manner that requires comment.

(a) ‘Induced transport tests’. Rebholz and Northrop (1993) prefer the term ‘tracer perturbation’ (Albery and Knowles, 1987), when these tests are applied to enzyme reactions. This may not, however, convey their full significance. Thus, considering:

Figure 1 Minimal homioiketic models

(1) shows the minimal scheme for an enzyme catalysing an S-to-P reaction. E and F are two forms of the free enzyme; X is the enzyme–substrate complex (Britton, 1973). The interconversion of F and E is not shown. (2) shows an alternative minimal homioiketic mechanism. k_{+1} etc. are rate constants; K_S and K_P are equilibrium constants.

(i) ‘induced counter-transport’, Widdas (1952) pointed out that, with a ‘mobile carrier’ mechanism, a substrate passing through a membrane down a free-energy gradient should cause the movement of an alternative substrate in the opposite direction against its free-energy gradient; the movement of the alternative substrate (which may, simply, be labelled substrate) thus involves the transfer of free energy released by the substrate moving down its free-energy gradient to the alternative substrate;

(ii) ‘induced co-transport’, some permeability mechanisms cause the alternative substrate to move in the same direction as the substrate, a process again involving the transfer of free energy (Britton, 1963).

Although the terms ‘induced counter-transport’ and ‘induced co-transport’ may be unfamiliar to enzymologists, they have the advantage that they emphasize the transfer of free energy. This transfer greatly restricts the mechanisms that can be involved.

(b) ‘Fluxes’. It is usual to analyse permeability data obtained with tracers in terms of unidirectional fluxes. If two chambers, A and B, are separated by a membrane, the unidirectional flux of a solute from A to B is the number of individual molecules of solute, per unit time, leaving chamber A, and appearing in chamber B. The net flux from A to B is given by the difference in the unidirectional fluxes in the two directions. Data from enzyme reactions using labelled substrates may, similarly, be analysed in terms of unidirectional fluxes. Correspondingly, theoretical unidirectional fluxes may be calculated for enzyme mechanisms (Britton, 1966b). Induced transport is directly related to the ratio of the unidirectional fluxes in the two directions (see below). However, although Rebholz and Northrop (1993) refer to tracer perturbation, they make no mention of (unidirectional) fluxes, and do not point out that the terms in square brackets in their eqn. (1) [taken from eqn. (1) of Britton (1973)] represent these fluxes. Further, in relation to this equation, they comment that the α terms cancel, and that therefore α represents a combination of rate constants. This is only true if the net flux, alone, is considered: α may be measured by directly determining the unidirectional fluxes or by carrying out an induced transport test. Their later statement, that α can have two possible values, was previously noted by Britton (1973) for the circumstance in which only the net flux is measured. Theoretical fluxes for enzyme reactions, incidentally, are probably most simply calculated by the ‘partitioning’ method (Britton, 1964, 1966b, 1967a). This procedure gives solutions in compact form, and they have been published for most of the common mechanisms. Knowledge of the amounts of the enzyme species present that react with the (labelled) substrates is required. A further development of the partitioning technique may be useful in deriving these values (Britton, 1977).

(c) Flux ratios and induced transport. Any discussion of ‘induced transport’ (tracer perturbation) should refer to its relationship to the ratio of the forward to reverse fluxes. If there is no transfer of free energy, of the type mentioned above in relation to induced transport, then, for an S-to-P reaction the flux ratio must obey the Independence Relationship even when a

net reaction is taking place (Britton, 1963, 1965, 1966a). The relationship is given by the expression:

$$\frac{\text{Flux of S to P}}{\text{Flux of P to S}} = \frac{[S]}{[P]K}$$

where K is the equilibrium constant ($[S]/[P]$), and $[S]$ and $[P]$ are the concentrations (or, more strictly, the activities) of S and P.

A system obeying this relationship will not give rise to induced transport. [There are corresponding relationships for the ratios of the forward to reverse fluxes in multi-substrate multi-product reactions (Britton, 1965).]

Under conditions of net conversion of S into P, if:

$$\frac{\text{Flux of S to P}}{\text{Flux of P to S}} < \frac{[S]}{[P]K}$$

there will be a transfer of free energy that leads to 'induced counter-transport'. By analogy with permeability, the system may also be described as showing 'exchange diffusion'.

Alternatively, under conditions of net conversion of S into P, if:

$$\frac{\text{Flux of S to P}}{\text{Flux of P to S}} > \frac{[S]}{[P]K}$$

there will be a transfer of free energy giving rise to 'induced co-transport'. By analogy with permeability, the system may be described as showing the 'long-pore effect'.

The amounts of induced counter-transport and induced co-transport depend only on the deviation of the flux ratio from the Independence Relationship, and the calculation is described by Britton (1973) and Britton and Clarke (1968).

To emphasize the inter-disciplinary nature of isotopic studies, although the terms 'exchange diffusion' and 'counter-transport' arose from permeability studies, a very early and extreme example of the phenomenon was reported in the chemical field. Thus, Bender (1951) found, during the alkaline hydrolysis of esters, extensive incorporation of ^{18}O from H_2O into the carbonyl oxygen of the ester: this was attributed to a 1:1 exchange process involving a tetrahedral intermediate complex. The converse of 'exchange diffusion', the 'long-pore effect', was observed in a study of the passive movement of K^+ through the membrane of the squid axon (Hodgkin and Keynes, 1955). This was regarded

as evidence for 'long-pores' in the membrane, in which at any one instant 2–3 K^+ ions are present. These K^+ ions are constrained to move in single file, and, if each 'step' consists of a K^+ moving into a pore, with a corresponding K^+ leaving at the other end, then 2–3 'steps' are necessary for a labelled K^+ ion to traverse the membrane. Considerably later, the 'long-pore effect' was demonstrated in the chemical field by showing 'induced co-transport' of ^{32}P -labelled substrates, in studies on the phosphoglucomutases and -glyceromutases (Britton, 1967b; Britton and Clarke, 1968, 1972; Clarke and Britton, 1974; Britton et al., 1972, 1973). With these enzymes [apart from wheat-germ phosphoglyceromutase, which has a different mechanism (Britton et al., 1971)], the active enzyme is a phosphoenzyme. During the reaction, the phosphate group of the phosphoenzyme is transferred to the substrate, and the phosphoenzyme is regenerated in the same turnover, by transfer of a phosphate group from the substrate. Thus, to transfer a labelled phosphate group from the substrate to the product, two molecules of substrate must react sequentially with the enzyme, to give two molecules of product. The enzyme mechanism is thus exactly analogous to that of the 'long-pore' of Hodgkin and Keynes (1955).

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- Albery, W. J. and Knowles, J. R. (1987) *J. Theor. Biol.* **124**, 137–171
 Bender, L. (1951) *J. Am. Chem. Soc.* **73**, 1626–1629
 Britton, H. G. (1963) *Nature (London)* **198**, 190–191
 Britton, H. G. (1964) *J. Physiol. (London)* **170**, 1–20
 Britton, H. G. (1965) *Nature (London)* **205**, 1323–1324
 Britton, H. G. (1966a) *Nature (London)* **209**, 296
 Britton, H. G. (1966b) *Arch. Biochem. Biophys.* **117**, 167–183
 Britton, H. G. (1967a) *Arch. Biochem. Biophys.* **121**, 522
 Britton, H. G. (1967b) *Biochem. J.* **103**, 7P–8P
 Britton, H. G. (1973) *Biochem. J.* **133**, 255–261
 Britton, H. G. (1977) *Biochem. J.* **161**, 516–526
 Britton, H. G. and Clarke, J. B. (1968) *Biochem. J.* **110**, 161–179
 Britton, H. G. and Clarke, J. B. (1972) *Biochem. J.* **130**, 397–410
 Britton, H. G., Carreras, J. and Grisolia, S. (1971) *Biochemistry* **10**, 4522–4533
 Britton, H. G., Carreras, J. and Grisolia, S. (1972) *Biochemistry* **11**, 3008–3014
 Britton, H. G., Carreras, J. and Grisolia, S. (1973) *Eur. J. Biochem.* **36**, 495–513
 Clarke, J. and Britton, H. G. (1974) *Biochem. J.* **134**, 453–461
 Cornish-Bowden, A. (1994) *Biochem. J.* **301**, 621–623
 Hodgkin, A. L. and Keynes, R. D. (1955) *J. Physiol. (London)* **128**, 61–88
 Rebholz, K. L. and Northrop, D. B. (1993) *Biochem. J.* **296**, 355–360
 Widdas, W. F. (1952) *J. Physiol. (London)* **118**, 23–29