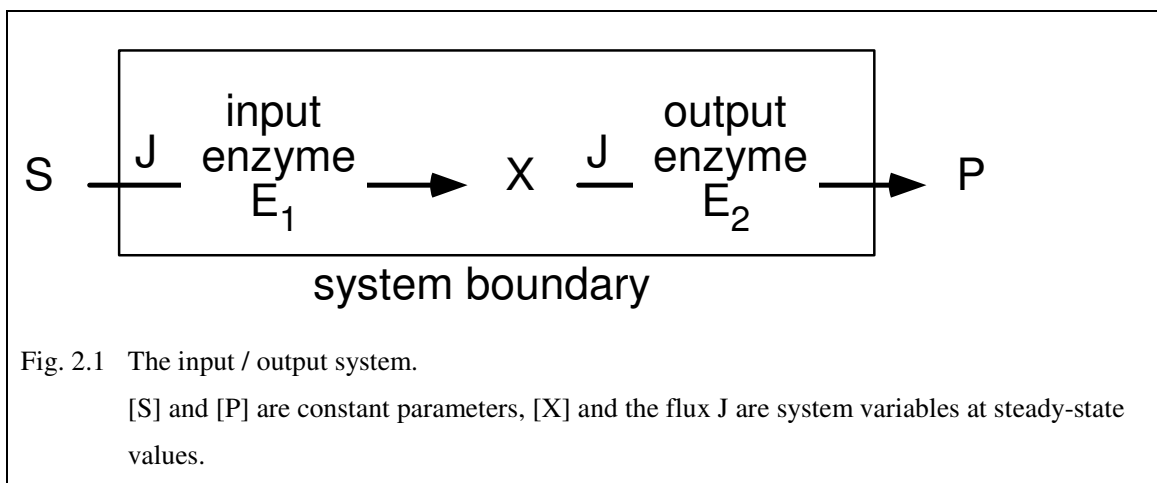


Chapter 2 Interplay between enzymes

§ 2.1 The input / output system

Consider a system consisting of two simple enzymes that act consecutively. The first enzyme (the input) converts a substrate S into intermediate compound X, the second enzyme (the output) converts X to the product P (see Fig. 2.1).



Consider the system at steady state, where X is produced at the same rate at which it is consumed. We use the boundary condition that [S] and [P] are constant as well, so effectively we have these two concentrations not as variables but as parameters. This system has *system properties* that are not attributable to the individual enzymes: the steady-state concentration of the intermediate [X], and the net flux through the system (equal to the rates of production and consumption of X).

An important question is what is the relation in such a system between the values of system variables such as [X] and J, and properties of the reactions (S, K_{eq} , P) and enzymes (K_M 's, V_{max} 's). To answer this question, one first has to consider the relationships between the enzymes, X and J.

In kinetic terms, it is expected that [X] affects the rates of the reactions catalysed by E_1 and E_2 (e.g. check out Eq. 1.8, with X acting as substrate or as product). Without any information about the precise kinetics of an enzyme-catalysed reaction, one can quantify the effect of an intermediate (substrate, product, inhibitor, activator) on the reaction rate as an *elasticity constant*, defined as:

$$\epsilon_{X_j}^i = \frac{\left(\frac{\partial v_i}{v_i}\right)}{\left(\frac{\partial X_j}{X_j}\right)} \quad (\text{Eq. 2.1})$$

This is the elasticity of the rate of enzyme-catalysed reaction i towards intermediate X_j . Note that partial derivatives are used; only the concentration of the intermediate is changed, all other intermediates are kept at their steady-state values. A second thing is to realize that the *value* of the elasticity coefficient depends on the exact steady state where it is determined. This is most easily seen when e.g. from Eq. 1.8, expressions for the elasticity of the enzyme with respect to [S] or to [P] are derived. Differentiation of this equation yields expressions for

the elasticity coefficients that not only depend on the enzyme parameters (K_M 's and V_{\max} 's) but also on the (steady-state values of) the variables [S] and [P]. Finally, the coefficient is a ratio between relative changes (∂ something / something). This assures the coefficient is dimensionless, and it can be shown mathematically that this is equivalent to using logarithmic variables ($\partial \ln$ something).

The next step is to define a measure of the effect that the enzymes have on the total flux. This is quantified with *flux control coefficients*, defined as:

$$C_{J_i}^J = \frac{\left(\frac{\partial J}{J}\right)}{\left(\frac{\partial J_i}{J_i}\right)} = \frac{\left(\frac{\partial J}{J}\right)}{\left(\frac{\partial e_i}{e_i}\right)} \quad C_i^J = \frac{\left(\frac{\partial J}{J}\right)}{\left(\frac{\partial v_i}{v_i}\right)} = \frac{\left(\frac{\partial J}{J}\right)}{\left(\frac{\partial e_i}{e_i}\right)}$$

(Eq. 2.2)

The true definition deals with the effect of a change of a local rate v_i on a system flux J , but as for an enzyme-catalysed reaction the flux generally is proportional to the enzyme concentration, often e_i is used instead of v_i .

A measure of the effect an enzyme has on an intermediate concentration is the *concentration control coefficient*:

$$C_i^{X_j} = \frac{\left(\frac{\partial X_j}{X_j}\right)}{\left(\frac{\partial v_i}{v_i}\right)} = \frac{\left(\frac{\partial X_j}{X_j}\right)}{\left(\frac{\partial e_i}{e_i}\right)} \quad (\text{Eq. 2.3})$$

Control coefficients for other system properties can be defined in a similar manner.

§ 2.2 Metabolic control analysis (MCA)

Metabolic control analysis uses elasticity coefficients and control coefficients to describe behaviour of metabolic systems at steady state. It can be shown that there are a number of relationships (MCA theorems) that relate these coefficients to each other. All theorems are derived from the idea that when a number of quantities are changed in a system, the response of the system can be calculated as a linear combination of changes that you would get when these quantities are changed one by one. The derivations apply to essentially extensive networks of intermediates X_j , connected by fluxes J_i (represented by enzyme concentrations e_i).

Intermezzo 3: derivation of MCA theorems

Figure 5 shows how linear approximations can be used to describe a change in a function of many variables (Euler's expression). We will use this expression to derive the MCA theorems. The trick is to choose the changes (between two steady states) and the variables in a clever way. To use the elasticity and control coefficients as defined, logarithms of functions and variables will be used. The equivalent Euler's expression will be:

$$d \ln F(\ln x, \ln y, \dots) = \left(\frac{\partial \ln F}{\partial \ln x} \right) d \ln x + \left(\frac{\partial \ln F}{\partial \ln y} \right) d \ln y + \dots \quad \text{or}$$

$$\frac{dF(x, y, \dots)}{F(x, y, \dots)} = \left(\frac{\frac{\partial F}{\partial x}}{F} \right) dx + \left(\frac{\frac{\partial F}{\partial y}}{F} \right) dy + \dots \quad (\text{Eq. 2.4})$$

Below we will derive MCA theorems for fluxes and concentrations.

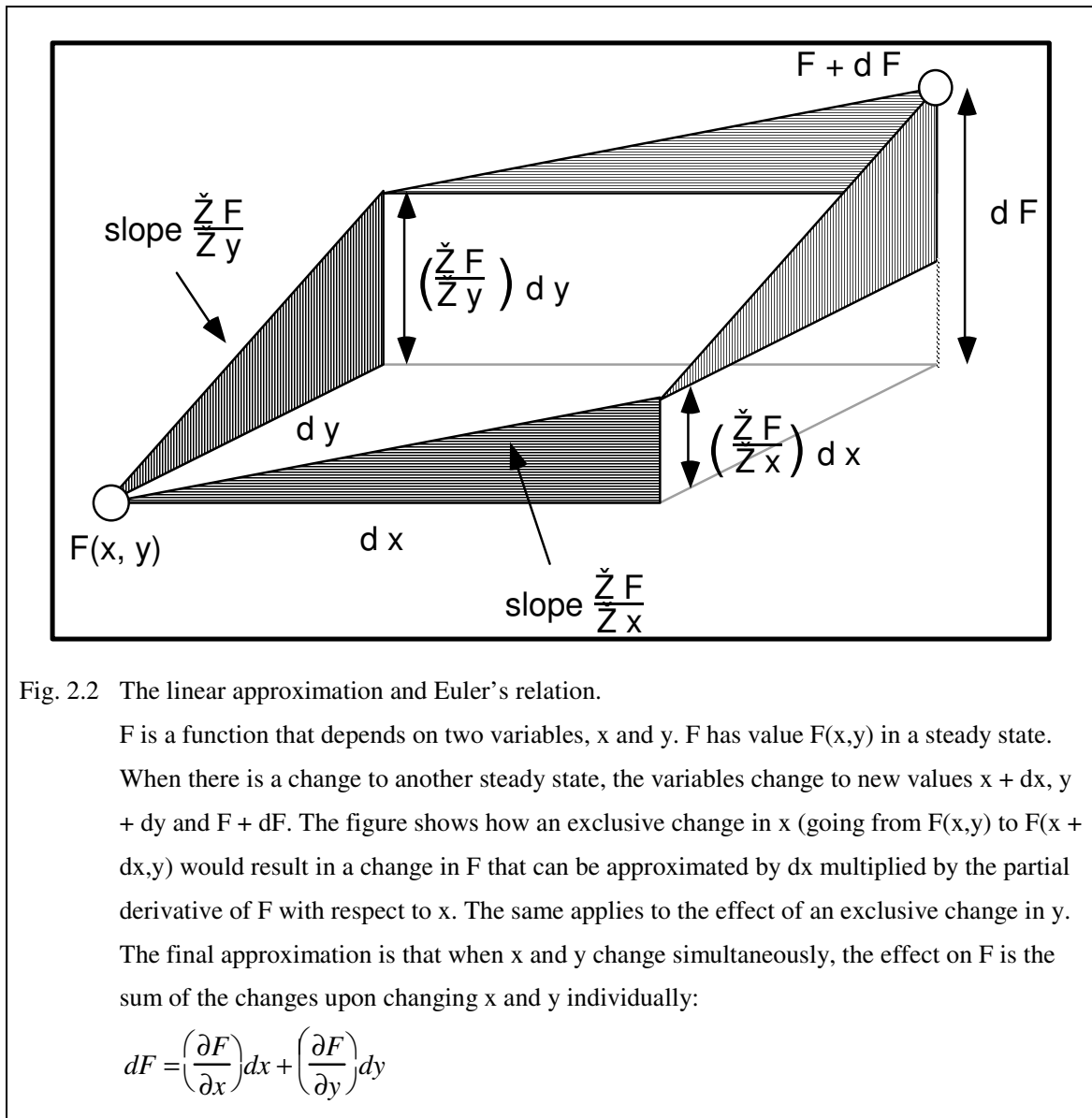


Fig. 2.2 The linear approximation and Euler's relation.

F is a function that depends on two variables, x and y . F has value $F(x, y)$ in a steady state.

When there is a change to another steady state, the variables change to new values $x + dx$, $y + dy$ and $F + dF$. The figure shows how an exclusive change in x (going from $F(x, y)$ to $F(x + dx, y)$) would result in a change in F that can be approximated by dx multiplied by the partial derivative of F with respect to x . The same applies to the effect of an exclusive change in y . The final approximation is that when x and y change simultaneously, the effect on F is the sum of the changes upon changing x and y individually:

$$dF = \left(\frac{\partial F}{\partial x} \right) dx + \left(\frac{\partial F}{\partial y} \right) dy$$

(a) The summation theorems

The function is a flux J , the quantities on which the flux depends are the enzyme concentrations e_i (c.f. the definition of the flux control coefficients). Euler's relation is

$$\frac{dJ}{J} = \sum_i \left(\frac{\partial J}{J} \right)_{e_i} \frac{de_i}{e_i} = \sum_i C_i^J \frac{de_i}{e_i}$$

Change all parameters e_i such that for all i : $\frac{de_i}{e_i} = \alpha$

The steady state is maintained, and all fluxes change by the same factor.

$$\frac{dJ}{J} = \sum_i C_i^J \frac{de_i}{e_i} = \sum_i C_i^J \alpha = \alpha$$

From this follows the *summation theorem for fluxes*:

$$\sum_i C_i^J = 1 \tag{Eq. 2.5}$$

The function is a intermediate concentration X_k , Euler's relation is

$$\frac{dX_k}{X_k} = \sum_i \left(\frac{\partial X_k}{X_k} \right)_{e_i} \frac{de_i}{e_i} = \sum_i C_i^{X_k} \frac{de_i}{e_i}$$

again, $\frac{de_i}{e_i} = \alpha$

The steady state is maintained, and all intermediate concentrations remain unchanged:

$$\frac{dX_k}{X_k} = \sum_i C_i^{X_k} \frac{de_i}{e_i} = \sum_i C_i^{X_k} \alpha = 0$$

From this follows the *summation theorem for concentrations*:

$$\sum_i C_i^{X_k} = 0 \tag{Eq. 2.6}$$

(b) The connectivity theorems

The function is a flux J_i , the changing quantities on which this flux depends are the enzyme concentration e_i and the intermediate concentration X_k . Eulers relation of choice is:

$$\frac{dJ_i}{J_i} = \left(\frac{\partial J_i}{J_i} \right)_{e_i} \left(\frac{de_i}{e_i} \right) + \left(\frac{\partial J_i}{J_i} \right)_{X_k} \left(\frac{dX_k}{X_k} \right)$$

Pick a concentration X_k , and change X_k such that $\frac{dX_k}{X_k} = \alpha$

Then change all e_i such that $\frac{de_i}{e_i} = -\alpha \varepsilon_{X_k}^i$

Because J_i is proportional to e_i , $\left(\begin{array}{c} \frac{\partial J_i}{\partial e_i} \\ J_i \\ e_i \end{array} \right) = 1$ it follows that

$$\frac{dJ_i}{J_i} = \left(\frac{de_i}{e_i} \right) + \left(\begin{array}{c} \frac{\partial J_i}{\partial X_k} \\ J_i \\ X_k \end{array} \right) \left(\frac{dX_k}{X_k} \right) = -\alpha \varepsilon_{X_k}^i + \varepsilon_{X_k}^i \alpha = 0$$

The fluxes don't change, so the steady state is maintained. Substitution into the Euler expression for fluxes yields:

$$\frac{dJ}{J} = \sum_i C_i^J \frac{de_i}{e_i} = \sum_i -\alpha \varepsilon_{X_k}^i C_i^J = 0$$

From this follows the *connectivity theorem for fluxes*:

$$\sum_i \varepsilon_{X_k}^i C_i^J = 0 \quad (\text{Eq. 2.7})$$

Note that every intermediate X_k generates a different connectivity theorem. Substitution into Euler's expression for concentration X_n yields:

$$\frac{dX_n}{X_n} = \sum_i -\alpha \varepsilon_{X_k}^i C_i^{X_n} = \alpha \delta_{kn}$$

In this expression, $\delta_{kn} = 0$ when $k \neq n$ (the fluxes don't change, so other intermediate concentrations don't change), $\delta_{kn} = 1$ when $k = n$.

From this follows the *connectivity theorem for concentrations*:

$$\sum_i \varepsilon_{X_k}^i C_i^{X_n} = -\delta_{kn} \quad (\text{Eq. 2.8})$$

In addition to summation and connectivity theorems, understanding of complicated networks that involve branching and cyclic structures sometimes requires extra theorems. Below is a derivation of branching and cycle theorems.

(c) The branching and cycle theorems

Select a stretch of n fluxes, that either connects in a circle (upper part of Fig. 2.3) or starts and ends at a source or sink (lower part of Fig. 2.3). Sources and sinks refer to concentrations that are part of the boundary conditions of the metabolic network, and consequently constant (like S and P in Fig. 2.1). This way it is possible to modify two fluxes at each node while maintaining the steady-state of the system as a whole. Obviously, each node may be the origin of more fluxes than the two that are part of the stretch.

The fluxes J_i have a sign that reflects their direction: e.g. $+(J_i > 0)$ from left to right and $-(J_i < 0)$ from right to left, in Fig. 2.3.

Applied modification of fluxes: $\frac{dJ_i}{J_i} = \alpha_i$

Maintaining the steady state at node i requires that $dJ_{i-1} = dJ_i$, so that

$$dJ_i = \alpha_i J_i = c \text{ (constant) or } \alpha_i = \frac{c}{J_i}$$

As a consequence, $\frac{dJ_i}{J_i} = \frac{c}{J_i}$ and $\frac{dX_j}{X_j} = 0$

within the stretch shown in Fig. 2.3. Outside this stretch, $\frac{dX_j}{X_j} = 0$ and $\frac{dJ_i}{J_i} = 0$.

Control exerted on system variable Y (a function of $\{X_i\}$ and / or $\{J_i\}$):

$$\frac{dY}{Y} = \sum_i \alpha_i C_i^Y = q$$

Y is a function of $\{X_i\}$ and / or $\{J_i\}$, so that q is function of c as soon as the dJ_i of the scheme ("inside stretch") are involved.

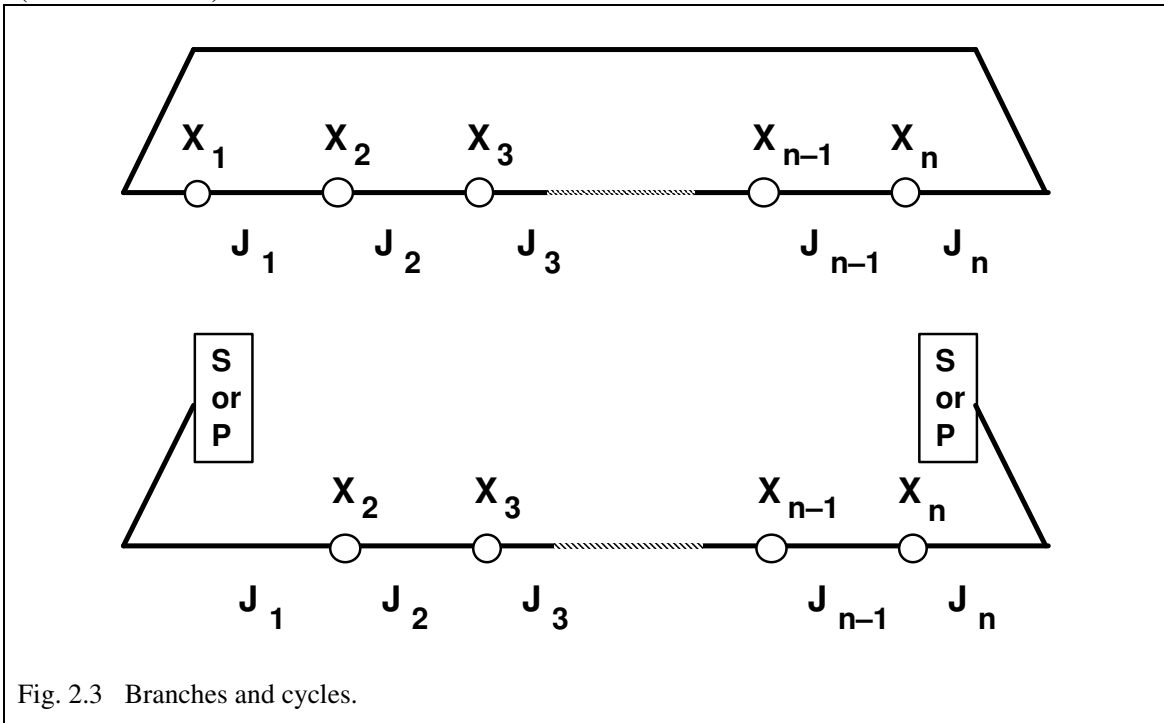


Fig. 2.3 Branches and cycles.

$$\Rightarrow \sum_i \frac{C_i^Y}{J_i} = \frac{q(c)}{c}$$

We will apply this expression to generate branching and cycle theorems for fluxes and concentrations (to supplement the summation and connectivity theorems above), and to show the generality of the approach we will also derive theorems for flux ratios (that are useful system properties in non-linear metabolic networks).

flux control (flux k inside stretch):

$$q = \frac{c}{J_k} \quad \rightarrow \quad \sum_i \frac{C_i^{J_k}}{J_i} = \frac{1}{J_k} \quad (\text{Eq. 2.9})$$

flux control (flux k outside stretch):

$$q = 0 \quad \rightarrow \quad \sum_i \frac{C_i^{J_k}}{J_i} = 0 \quad (\text{Eq. 2.10})$$

concentration control:

$$q = 0 \quad \rightarrow \quad \sum_i \frac{C_i^{X_k}}{J_i} = 0 \quad (\text{Eq. 2.11})$$

With a flux ratio: $r_{km} = \frac{J_k}{J_m}$ flux ratio control is:

both fluxes k and m inside stretch:

$$q = \frac{c}{J_k} - \frac{c}{J_m} \quad \rightarrow \quad \sum_i \frac{C_i^{r_{km}}}{J_i} = \frac{1}{J_k} - \frac{1}{J_m} \quad (\text{Eq. 2.12})$$

flux k inside and flux m outside stretch:

$$q = \frac{c}{J_k} \quad \rightarrow \quad \sum_i \frac{C_i^{r_{km}}}{J_i} = \frac{1}{J_k} \quad (\text{Eq. 2.13})$$

flux k outside and flux m inside stretch:

$$q = -\frac{c}{J_m} \quad \rightarrow \quad \sum_i \frac{C_i^{r_{km}}}{J_i} = -\frac{1}{J_m} \quad (\text{Eq. 2.14})$$

both fluxes k and m outside stretch:

$$q = 0 \quad \rightarrow \quad \sum_i \frac{C_i^{r_{km}}}{J_i} = 0 \quad (\text{Eq. 2.15})$$

Taking into account Fig. 2.3, there is no difference between *branching and cycle theorems*.

Table 2.I Summation and connectivity theorems for the two-enzyme input / output system of Fig. 2.1.

	Summation theorem	Connectivity theorem
Flux J	$C_{input}^J + C_{output}^J = 1$	$\epsilon_X^{input} C_{input}^J + \epsilon_X^{output} C_{output}^J = 0$
Intermediate X	$C_{input}^X + C_{output}^X = 0$	$\epsilon_X^{input} C_{input}^X + \epsilon_X^{output} C_{output}^X = -1$

§ 2.3 Applications

From the summation theorem, it is immediately clear that control of a flux may be distributed among a number of enzymes. Provided that the corresponding control coefficient is non-zero, a change in the amount of enzyme will immediately have effect on the overall flux. Generally, there is no single *rate-limiting step* in a metabolic pathway.

To find out whether control (of a flux) can be limited to a single or a small number of enzymes, we will consider the input / output system. The relations between the flux control coefficients are (Table 2.I):

$$C_{input}^J + C_{output}^J = 1$$

$$\varepsilon_X^{input} C_{input}^J + \varepsilon_X^{output} C_{output}^J = 0$$

When the kinetics of the input- and output enzyme are known (K_{eq} , K_S , K_P and V_S), for any steady state ε_X^{input} and ε_X^{output} can be calculated (by differentiating Eq. 1.8, and inserting the fixed [S] and [P], and the calculated $[X]_{steady\ state}$). Using the above set of linear equations, the two flux control coefficients can be calculated.

Now assume that the input reaction (conversion of S to X, catalysed by E_{input}) is irreversible, and product insensitive. This happens when the K_M for X is extremely large, see § 1.4. There is no dependence of the input rate on [X], and thus $\varepsilon_X^{input} = 0$. Even without using higher mathematics, it is obvious that then $C_{output}^J = 0$ and $C_{input}^J = 1$: all flux control resides at the input enzyme, which then is rate limiting.

The same reasoning can be applied to a longer linear pathway. If one can divide the pathway in two parts in such a way that the first path is completely insensitive to the intermediates in the second part (all relevant elasticity coefficients are 0), all flux control resides in the first part.

This effect of product-insensitivity is an extreme case of the principle that elasticity coefficients have a sort of "inverse relation" to flux control. Solving the set of equations for the input / output system yields for the flux control coefficients:

$$C_{input}^J = \frac{\varepsilon_X^{output}}{\varepsilon_X^{output} - \varepsilon_X^{input}} \qquad C_{output}^J = \frac{-\varepsilon_X^{input}}{\varepsilon_X^{output} - \varepsilon_X^{input}} \quad (\text{Eq. 2.16})$$

Increasing ε_X^{output} increases C_{input}^J , and thus decreases C_{output}^J . Note that ε_X^{input} is negative (increasing [X] decreases the input flux) and ε_X^{output} is positive (increasing [X] increases the output flux). This also assures that $\varepsilon_X^{input} \neq \varepsilon_X^{output}$, and we can actually calculate the flux control coefficients, and they have a positive value.

The only kind of elasticity we have discussed up to now, is that of an enzyme towards its substrates and products. However, metabolites that do not take part in the reaction catalysed by the enzyme, may act as inhibitor or activator of the enzyme. In terms of MCA this means the elasticity coefficient of an enzyme towards an inhibitor has a *negative* value, and towards an activator has a *positive* value. When the inhibitor or activator is a precursor of a substrate

of the reaction, this is called *feed-forward* regulation; when the inhibitor or activator is produced (after a number of steps) from a product of the reaction, this is called *feed-back* regulation.

Different types of regulation are best illustrated with the simple pathways in Fig. 2.4. In MCA, the regulatory effects can be described in terms of the elasticity coefficients in the set of equations:

$$C_{e_1}^J + C_{e_2}^J + C_{e_3}^J = 1 \quad (\text{summation})$$

$$\varepsilon_{X_1}^{e_1} C_{e_1}^J + \varepsilon_{X_1}^{e_2} C_{e_2}^J + \varepsilon_{X_1}^{e_3} C_{e_3}^J = 0 \quad (\text{connectivity around } X_1)$$

$$\varepsilon_{X_2}^{e_1} C_{e_1}^J + \varepsilon_{X_2}^{e_2} C_{e_2}^J + \varepsilon_{X_2}^{e_3} C_{e_3}^J = 0 \quad (\text{connectivity around } X_2)$$

In the absence of regulation, $\varepsilon_{X_1}^{e_3} = \varepsilon_{X_2}^{e_1} = 0$. With positive feed-forward regulation of e_3 by X_1 , $\varepsilon_{X_1}^{e_3} > 0$. Negative feed-forward regulation (feed-forward inhibition) means $\varepsilon_{X_1}^{e_3} < 0$; positive and negative feed-back correspond to $\varepsilon_{X_2}^{e_1} > 0$ and $\varepsilon_{X_2}^{e_1} < 0$, respectively.

It is a bit difficult to see what effect these regulations have on the distribution of flux control in this pathway. Let us consider feed-back regulation of e_1 by X_2 ($\varepsilon_{X_1}^{e_3} = 0$, $\varepsilon_{X_2}^{e_1} \neq 0$). Let's further assume that enzymes have a positive elasticity towards substrates ($\varepsilon_{X_1}^{e_2}, \varepsilon_{X_2}^{e_3} \geq 0$) and a negative one with respect to products ($\varepsilon_{X_1}^{e_1}, \varepsilon_{X_2}^{e_2} \leq 0$). The flux control coefficients are:

$$C_{e_1}^J = \frac{A}{A + B + C - D\varepsilon_{X_2}^{e_1}} \quad C_{e_2}^J = \frac{B}{A + B + C - D\varepsilon_{X_2}^{e_1}} \quad C_{e_3}^J = \frac{C - D\varepsilon_{X_2}^{e_1}}{A + B + C - D\varepsilon_{X_2}^{e_1}}$$

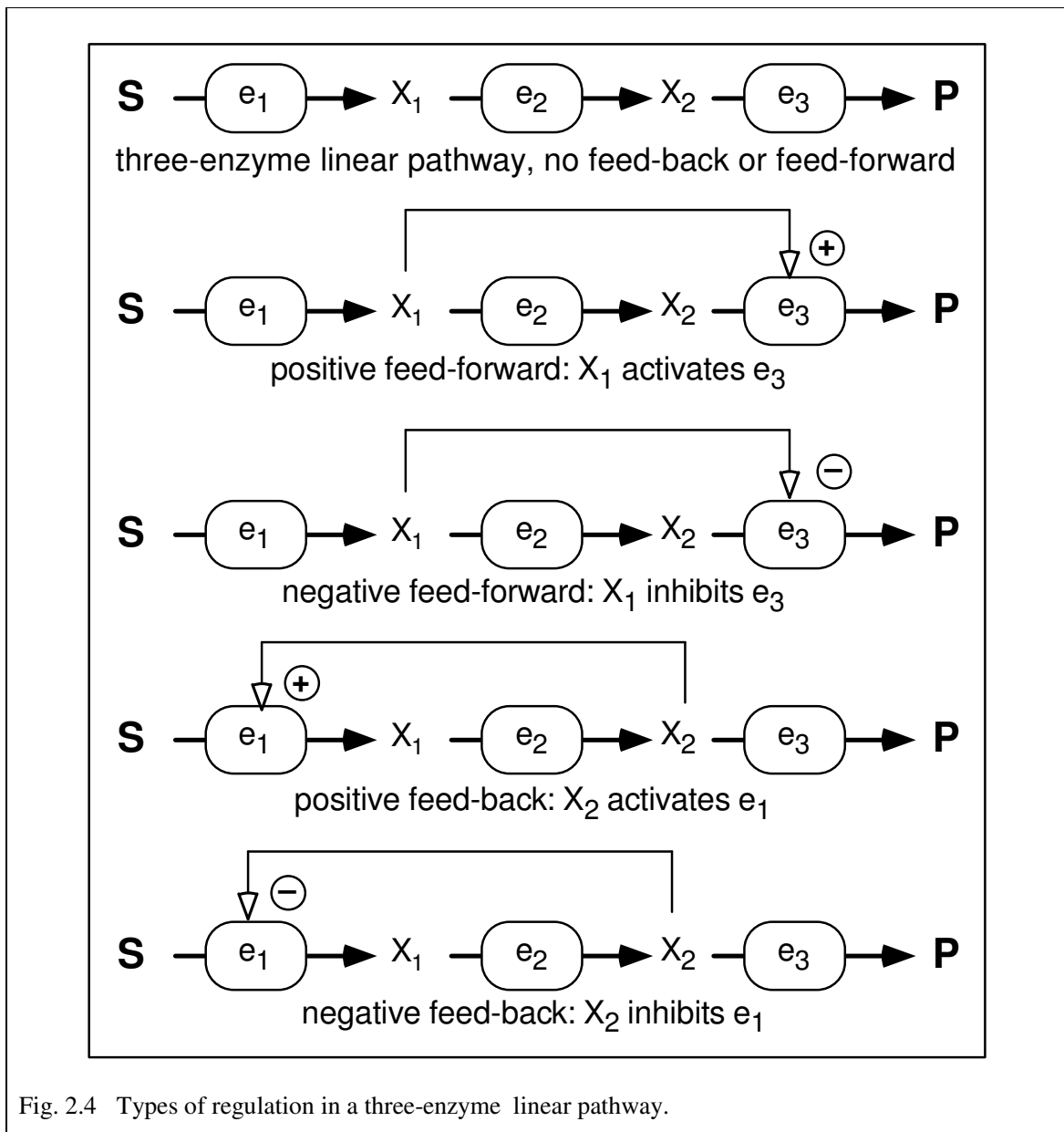


Fig. 2.4 Types of regulation in a three-enzyme linear pathway.

A, B, C and D are all positive combinations of the other four elasticity coefficients. From these expressions it follows that, compared to the case where there is no regulation ($\varepsilon_{X_2}^{e_1} = 0$), positive feed-back ($\varepsilon_{X_2}^{e_1} > 0$) increases C_1 and C_2 and decreases C_3 . In other words, positive feedback shifts flux control to the enzyme that is regulated (e_1). On the other hand, feed-back inhibition ($\varepsilon_{X_2}^{e_1} < 0$) decreases C_1 (and C_2) and shifts control away from e_1 . This effect is quite general, and not restricted to the system of Fig. 2.4). In a similar way it can be shown that positive feed-forward by X_1 increases control by e_3 , and negative feed-forward decreases control by e_3 .

The strength of MCA is that one can formulate such statements about systems of reactions without need for exhaustive kinetic description of the kinetics of the individual steps. Knowledge about the pattern of reactions, some common sense and only a little mathematics are sufficient.

Processes in living cells are not only affected by substances taking part in them, but sometimes also by external compounds. To analyse the effects of any compound on a system in steady state in terms of MCA, one can use response coefficients. These can be defined similarly to control coefficients (the affected property is a system property). The response of property Z on compound Y is defined as:

$$R_Y^Z = \frac{\left(\frac{\partial Z}{Z}\right)}{\left(\frac{\partial Y}{Y}\right)} \quad (\text{Eq. 2.17})$$

Elasticity coefficients (the affected property is a single rate) towards Y are defined as:

$$\epsilon_Y^i = \frac{\left(\frac{\partial v_i}{v_i}\right)}{\left(\frac{\partial Y}{Y}\right)} \quad (\text{Eq. 2.18})$$

Again, Euler's expression can be used to great effect to analyse the response of a system property in terms of the responses of individual processes.

§ 2.4 MCA in practice

One way to apply MCA to a metabolic system is to determine the control coefficients directly. This is done by measuring a (steady-state value of) a system property in response to modulation of a single step in the system. Ideally, this can be done by using an irreversible inhibitor that allows "elimination" of the target enzyme in a precise quantitative manner. However, specific, fast reacting, irreversible inhibitors are rare, so other solutions have been sought. One is to use a reversible inhibitor, and compare the effects of the inhibitor on the system, with the effects of the inhibitor on the enzyme in isolation. The latter effect then is translated into a fraction of the enzyme that is eliminated at given inhibitor concentration. A problem is here, that inhibition is dependent on the precise circumstances, and it is not always possible to make the conditions during the assay of the isolated enzyme sufficiently similar to those under which the enzyme operates in the complete system.

The modulation of individual processes required for experimental MCA is not always the result of intervention at the metabolic timescale (such as adding an inhibitor to a functioning cell). Modulation can be achieved as well by changing protein expression. A useful approach is to insert a site for an inducible promoter in front of the gene coding for an enzyme that catalysis the metabolic step of choice. This way, expression of the gene can be modulated by way of the promoter activity. Favourite tool in this approach is the lacZ promoter from *E. coli*, that can be induced by IPTG.

A totally different approach (the *top-down approach*) makes use of the MCA theorems to calculate control coefficients from elasticity coefficients (and, in case branching or cycles occur, fluxes). For e.g. the input / output system, Eq. 2.16 can be used to calculate the two flux control coefficients. To determine the required elasticity coefficients, again inhibitors are used to interfere with the system under study.

§ 2.5 Extensions

When studying metabolic systems, the huge number of interacting processes sometimes overwhelms one. Often it clearly is not feasible to formulate, and use the required number of MCA theorems that quantify control distribution in the system. There is a way out, however. It often is possible to divide a system in a limited number of subsystems or modules, that each may contain many enzymatic processes. These subsystems can be treated as single processes, with their subsystem elasticity coefficients and subsystem control coefficients. When more resolution is required, a subsystem can be studied further in terms of its own constituents. This *modular approach* to MCA can be used with great effect together with the top-down method to determine control coefficients. Below (§ 3.3 and further) we will discuss an example.

Up to now we have only considered control analysis at the metabolic level (MCA). At the metabolic timescale enzyme concentrations are assumed to be constant, and thus are parameters rather than variables. We have used this to great effect by misusing enzyme concentrations in our calculation of control coefficients (Eqs. 2.2 and 2.3). However, in the study of functioning of living cells changes in protein expression levels are frequently encountered and rather important. In this respect it is good to realize that not only does transcription determine what happens at the metabolic level, but that metabolite concentrations are important determinants of protein expression. Many metabolites are regulators of protein expression, switching genes on or off, or having more subtle effects on enzyme levels.

To accommodate these phenomena into a quantitative description of cellular behavior, MCA has been extended into *Hierarchical Control Analysis* (HCA), which takes into account different hierarchical levels of cell function, such as metabolic level, gene transcription- (DNA) and translation (RNA) level.

As a relatively simple example, again consider the input / output system of Fig. 2.1. Let's assume that the cell profits when the concentration of X is kept constant under many different conditions (homeostasis). In first approximation, this is taken care of by enzymes E_{input} and E_{output} . When we assume that the response coefficient of enzyme E_{input} to its product X is negative ($\epsilon_X^{E_{input}} < 0$), and that the response coefficient of enzyme E_{output} to its substrate X is positive ($\epsilon_X^{E_{output}} > 0$), it is easily seen that raising [X] will inhibit E_{input} and stimulate E_{output} with net effect lowering [X] again. This effect can be strengthened when X influences the level of the two enzymes. E.g., X could stimulate production of E_{output} , and inhibit production of E_{input} . This effect can be quantified by comparison of the control the two enzymes have on [X]. In the case without X effects on gene expression, one can derive from the theorems in Table 2.1:

$$C_{input}^X = \frac{1}{\epsilon_X^{output} - \epsilon_X^{input}} \qquad C_{output}^X = \frac{-1}{\epsilon_X^{output} - \epsilon_X^{input}} \quad (\text{Eq. 2.19})$$

When X inhibits expression of E_{input} , there is an elasticity coefficient $\epsilon_X^{E_{input} \text{ synthesis}} < 0$. Similarly, when X stimulates expression of E_{output} , there is an elasticity coefficient $\epsilon_X^{E_{output} \text{ synthesis}} > 0$. One can derive monstrous expressions for the control of [X] such as:

$$\text{input } O_{\text{input}}^X = \frac{1}{(\epsilon_X^{\text{output}} - \epsilon_X^{\text{input}}) + \left(\frac{\epsilon_X^{\text{output synthesis}}}{\epsilon_{\text{output}}^{\text{output degradation}} - \epsilon_{\text{output}}^{\text{output synthesis}}} \right)}$$

$$\text{output } O_{\text{output}}^X = \frac{-1}{(\epsilon_X^{\text{output}} - \epsilon_X^{\text{input}}) - \left(\frac{\epsilon_X^{\text{input synthesis}}}{\epsilon_{\text{input}}^{\text{input degradation}} - \epsilon_{\text{input}}^{\text{input synthesis}}} \right)}$$

The O 's are examples of *co-response coefficients*, that quantitate the ratio of the relative changes of the variables in the super- and subscripts at the right, when the variable of the left superscript is changed.

Because of the signs of the six additional elasticity coefficients appearing in these expressions, the absolute value of these co-response coefficients is smaller than the absolute value of the corresponding concentration control coefficients. In other words, [X] becomes less sensitive to changes in input or output: homeostasis.