HYSTERESIS WITHOUT AUTOCATALYSIS: SIMPLE ENZYME SYSTEMS AS POSSIBLE BINARY MEMORY ELEMENTS

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Models with hysteresis play a considerable role in the theory of control processes on the genetic level, e.g. induction and differentiation as given by Monod and Jacob [1], but are also used to offer plausible possibilities for fundamental elements of thinking like memory, especially short-term memory.

In homogeneous reaction kinetics such models were introduced by Spangler and Snell [2, 3] who assume, like Monod and Jacob, cross-inhibition by the products of two enzyme reactions. An analytically simpler model was treated by Edelstein [4, 5], who used enzyme catalysis preceded by an autocatalytic step. An even simpler possibility of obtaining multiple steady states and a hysteresis loop without autocatalysis, which is seldom attained directly but which can be brought about by indirect mechanisms with positive feedback, exists in using forward inhibition instead.

O'Neill et al. [6] and O'Neill [7] stated that inhibition by an excess of substrate, which is shown by many enzymes [8], suffices under certain conditions to cause multiple steady states in open reaction systems. The same effect with the same type of reaction was found independently by Rössler [9] in a survey of basic circuits of fluid automata and was given without further treatment as one example of a resettable switch.

We chose this type of reaction for a deeper theoretical investigation, because i) it seems to be the simplest one yet known with the desired effect, ii) it utilizes an effect often found in enzyme kinetics and has no exotic additional requirements, and iii) it allows for a drastic reduction of parameters without loss of generality so that conditions of occurrence of the hysteresis effect can be shown with great clearness. Besides another completely isomorphous reaction type can be given.

According to the reaction scheme of fig. 1 we get for the reaction rates $\dot{X}_i \equiv dX_i/dt$ in dependence on the concentrations X_i

$$\dot{S} = k_{+1}R - (k_{-1} + k_{+5})S - k_{+2}S \cdot E + k_{-2}ES - k_{+4}S \cdot ES + k_{-4}ES_2,$$

$$\dot{E} = -k_{+2}S \cdot E + (k_{-2} + k_{+3})ES,$$

$$\dot{ES}_2 = k_{+4}S \cdot ES - k_{-4}ES_2, \quad ES = E_t - E - ES_2,$$

where E_t is the total amount of enzyme.

In the steady state(s) we are interested in, the rates become zero and by elimination of E, ES, and ES_2 we get the implicit cubic equation for S in the rather complicated form of

$$(k_{-1} + k_{+5})k_{+2}k_{+4}S^3 - (k_{+1}k_{+2}k_{+4}R - (k_{-1} + k_{+5})k_{+2}k_{-4})S^2 - (k_{+1}k_{+2}k_{-4}R - (k_{-1}k_{+5})(k_{-2} + k_{+3})k_{-4} - k_{+2}k_{+3}k_{-4}E_t)S - k_{+1}(k_{-2} + k_{+3})k_{-4}R = 0 \quad (1)$$

This can be drastically simplified without loss of generality by using the technique of introducing dimensionless variables and parameters. By defining

$\sigma = k_{+2}S/(k_{-2} + k_{+3})$,
$\rho = k_{+1}k_{+2}R/((k_{-1} + k_{+5})(k_{-2} + k_{+3}))$
$\epsilon = k_{+2}k_{+3}E_t/((k_{-1} + k_{+5})(k_{-2} + k_{+3}))$,

$\kappa = k_{+2}k_{-4}/(k_{+4}(k_{-2} + k_{+3}))$
eq. 1 is reduced to

$$\sigma^3 + \sigma^2(\kappa - \rho) + \kappa\sigma(1 + \epsilon - \rho) - \kappa\rho = 0, \qquad (2)$$

which contains only 3 instead of 10 parameters and as before, one variable. Only κ is fixed, ρ and ϵ contain concentrations and can thus be varied between 0 and the corresponding saturation concentrations of the pool substance R and the enzyme E, respectively.

As a cubic equation in σ, equation (2), can have three real positive roots in certain ranges of the parameters, the central one of which is then dynamically unstable, whilest the outer ones represent the stable states of the hysteresis loop, between which the system can be switched. Among the parameters ρ, ϵ and κ the influx ρ seems to be most likely to represent the input signal to which σ is the output response. The explicit function $\sigma = f(\rho)$ can be easily constructed, because the inverse function

$$\rho = f^{-1}(\sigma) = \sigma + \epsilon\sigma/(\sigma^2/\kappa + \sigma + 1) \qquad (3)$$

is unique.

Another system with second order inhibition by a side chain product of the substrate as indicated in fig. 2 is isomorphous with the former system, because its steady state equation is exactly the same as (2), if all variables and parameters of this system are primed (EI'_2 here corresponds to ES_2 there) and if the analogous dimensionless quantities are introduced save $\kappa' = k'^2_{+2}\, k'_{-4}\, k'^2_{+6}/((k'_{-2} + k'_{+3})^2 k'_{+4} k'^2_{+5}$. But this model will not be further pursued here separately.

A typical hysteresis loop for σ as a function of ρ with fixed parameters $\kappa = 1$ and $\epsilon = 100$ is given in fig. 3. For the inverse function $\rho = f^{-1}(\sigma)$ the points A, B, C represent a maximum, point of inflection, and minimum respectively, which are found by setting $\partial\rho/\partial\sigma$ and $\partial^2\rho/\partial\sigma^2$ equal to zero in the usual way. These conditions yield for the σ values of the extrema the implicit function

$$(\sigma^2 + \kappa\sigma + \kappa)^2 = \epsilon\kappa(\sigma^2 - \kappa) \qquad (4)$$

and

$$\sigma^3 = 3\kappa\sigma + \kappa^2 \qquad (5)$$

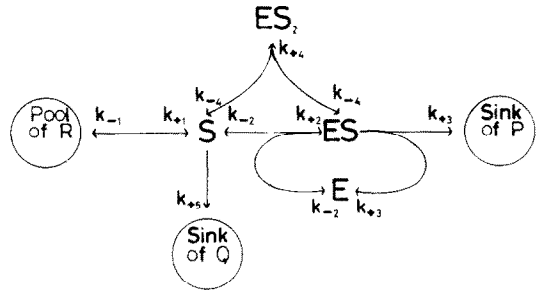

Fig. 1. Hysteresis system I with substrate inhibition of enzyme E.

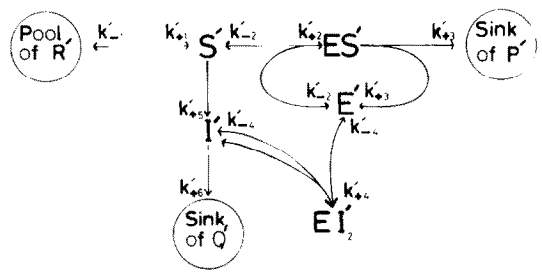

Fig. 2. Hysteresis system II with second order inhibition of enzyme E' by the intermediate I' of a side chain.

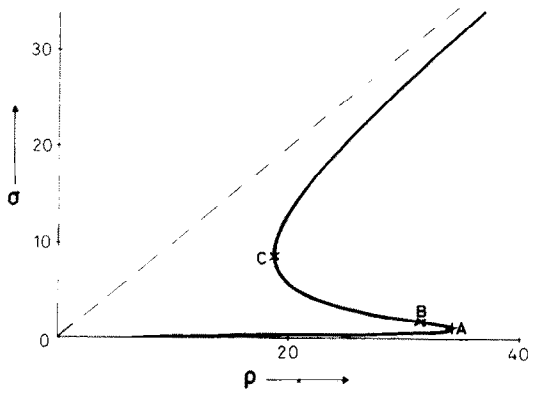

Fig. 3. Hysteresis loop $\sigma = f(\rho)$ as obtained from eq. (3) with $\kappa = 1$ and $\epsilon = 100$.

for the σ of the point of inflexion. The limiting case of a hysteresis loop for any ρ is given by the condition that the three points A, B, C coincide and represent a saddle point. In this case the common σ

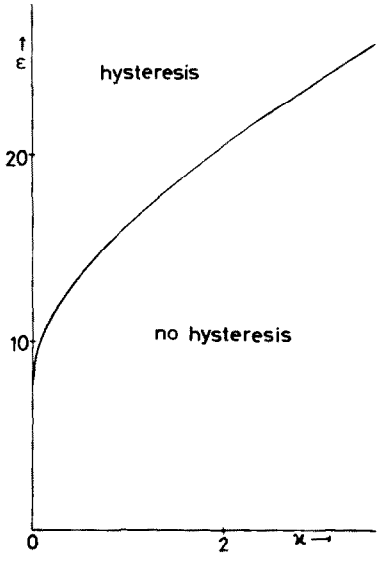

Fig. 4. Influence of the parameters κ and ϵ on the possibility of hysteresis for any ρ.

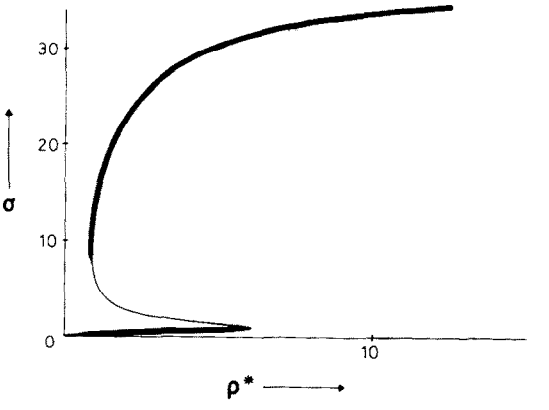

Fig. 5. Hysteresis loop as a reflected "Z" in case when the influx is regulated by a saturable additional enzyme E* (η = 40). The thick curves are the two quasi "quantum" states between which the system can be switched as a binary memory element.

can be eliminated from (4) and (5) yielding a complicated implicit function of ϵ and κ, which is a polynomial of sixth degree in ϵ and seventh degree in κ. The explicit function $\epsilon = g(\kappa)$ for this limiting case was evaluated numerically and is given in fig. 4. The minimum and the maximum of $\rho = f^{-1}(\sigma)$ and hence the hysteresis effect are the more pronounced the greater ϵ is above this limit.

The question arises whether these are purely theoretical results and whether there are real enzyme systems with their parameters in the necessary region. Unfortunately in no case given in the literature is there a complete set of parameters leading to κ and ϵ and in most cases it is not reported whether the substrate inhibition is according to the mechanism treated here†. The ratio, κ, of the dissociation constant of the complex ES_2 to the Michaelis constant K_m, should be rather small, but ϵ, which is E_t/K_m times the ratio of the rate constants of the catalysed to the uncatalysed reactions of S, should be fairly large.

Preliminary investigations of other mechanisms as given by Cleland [10] (ordered bi–bi, ping–pong, mixed type etc.) have shown that there are a few additional parameters left, but that the overall effect is much the same.

As shown in fig. 3 there is a relatively constant (with respect to σ) lower state, but the upper state grows eventually linearly with ρ. This upper state can be bent to a horizontal line, too, if the substrate is not restored by a spontaneous first order reaction, but is fed in controlled by a second enzyme E* with Michaelis constant K_m^*. In this case the term $k_{+1}R$ has to be replaced by

$$k_{+1}R\frac{E_t^*}{R+K_m^*} \quad .$$

If now k_{-1} is neglected and the dimensionless parameter

$$\eta = E_t^*\frac{k_{+1}k_{+2}}{k_{+5}(k_{-2}+k_{+3})}$$

† *Note added in proof*: H. Degn (Nature 217 (1968) 1047) reported experimental hysteresis shown with the oxidation of NADH by O_2 catalysed by peroxidase, which he interpreted at first as substrate inhibition by O_2, but in later publications (Biochim. Biophys. Acta 180 (1969) 29; J. Chem. Education 49 (1972) 302) as a complicated case of back activation.

is introduced, and if finally in (3) ρ is redefined as $\rho^* = R/K_m^*$, ρ has to be replaced by

$$\rho^* \frac{\eta}{\rho^* + 1}$$

and the inverse function is now

$$\rho^* = \frac{\sigma(\sigma^2/\kappa + \sigma + \epsilon + 1)}{(\eta - \sigma)(\sigma^2/\kappa + \sigma + 1) - \sigma\epsilon} \qquad (6)$$

This function has a singularity at a certain $\sigma = \sigma_{sat}$, which is the saturation value of σ for $\rho^* \to \infty$.

So the hysteresis loop is bent to a reflected "Z", there are virtually two "quantum" states between which the system is switched, as demonstrated in fig. 5 for $\kappa = 1$, $\epsilon = 100$ and $\eta = 40$.

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