Analysis and Simulation of Biochemical Systems

Volume 25

Organized by:
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INTRODUCTION

Thermodynamics and kinetics form an integral part of biochemistry. Yet, it was only recently that it became obvious that theoretical biochemistry has begun to play a major role among the various experimental fields of biochemistry. The development of theoretical biochemistry has evolved from the fact that several aspects of biochemistry are now gradually merging and beginning to show a consistent pattern of interrelationships. For the first time, the possibility of developing common viewpoints in the fields has opened up. We think of the fields of non-equilibrium thermodynamics and kinetics as seen in evolution, in allostericity and periodic reactions, simulation and data fitting, and also of the structure-function relationships aided by modern computer techniques.

It is difficult to date the birth of this discipline. It may have coincided with the EMBO course on computation in biochemistry (Edinburgh, 1968), or the appearance of the book on non-equilibrium thermodynamics in biophysics by A. Katchalsky and P.Curran (1965), the book on thermodynamic theory of structure, stability, and fluctuation by P.Glansdorff and I.Prigogine (1971), or the article on the self-organization of matter and the evolution of biological macromolecules by Manfred Eigen (1971). The book on temporal organization in cells by B.Goodwin (1963) should also be mentioned in this connection.

This development fully justified the decision of the organizers of the VIIIth Meeting of the Federation of European Biochemical Societies in Amsterdam in August of 1972 to include a symposium on the analysis and simulation of biochemical systems for the first time as part of an international congress. The organizers of this symposium tried to cover the subject by inviting authoritative scientists in the field of thermodynamics, kinetics, and computation of parameters and structures. In addition, a series of free communications dealing with various novel problems were presented.

This issue comprises articles collected from among both the invited contributions and the selected free communications. The papers summarize the major viewpoints from a number of laboratories and clearly illustrate the present situation in the field. The mixture finally in print may appear to be ripe or premature, and is probably far from equilibrium - and therefore even more interesting.

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DISSIPATIVE STRUCTURES AND THEIR APPLICATION TO BIOLOGY

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INTRODUCTION

I shall start my talk with a short summary of the work done by our group in Brussels since 1967.

The aim of our studies was essentially to develop a non-linear thermodynamics and to apply it to biological systems.

From a purely thermodynamical point of view the main findings are the establishment of conditions of stability of a thermodynamical system far from equilibrium.

It was soon realized that these new thermodynamical concepts are of interest for the understanding of certain aspects of biological order related both to the problems of the origin and evolution of prebiotic matter as well as to the problem of the maintenance of order in actual living organisms.

For a long time biological order has been a puzzle for scientists who tried to explain it on a physico-chemical basis without the intervention of "vitalistic" concepts. Biological systems are highly complex and ordered objects. They carry with them structures and functions acquired during a long evolution. On the other hand the maintenance of life, i.e. metabolism, synthesis and regulation imply a highly heteroge-

neous distribution of matter inside the cell through chemical reactions and active transport. Coherent behaviour is really the characteristic feature of biological systmes.

On the other hand, it is well known that a physico-chemical system left alone evolves towards an equilibrium state of maximum disorder. In an isolated system which cannot exchange energy and matter with the surroundings this tendency is expressed in terms of a function of the macroscopic state of the system : the entropy S . Thus the celebrated second law of thermodynamics states that the entropy increases monotonically till its maximum value. Therefore in an isolated system the formation of law entropy structures is not possible (1).

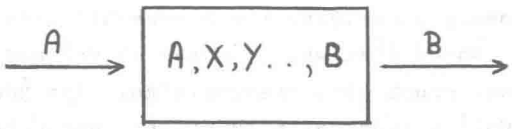
Let us consider now equilibrium and non equilibrium systems exchanging energy with a thermostat or matter with a reservoir of given chemical potential. It can be shown that in such systems there exists a possibility of occurence of low entropy structures like crystals.

Equilibrium phase transitions however can hardly explain the formation of biological structures. The probability, as calculated by the usual Boltzmann factor that at ordinary temperature a great number of molecules be assembled to give rise to the highly ordered structures and coordinated functions characterizing a biological system is vanishingly small. Thus the idea of spontaneous genesis of life in its present form seems a highly improbable event even for the period of bil lions of years of prebiotic evolution. We conclude that equilibrium statistical mechanics and thermodynamics cannot remove the " paradox" of biological order (second law).

The foregoing conclusions suggest that one should ask how biological structures may appear in physico-chemical systems maintained far from thermodynamic equilibrium? This question has been investigated extensively by our group.

It has been shown tht order may indeed appear in far from equilbrium situations when supplementary conditions are satisfied. (2),(3),(4). We here shall summarize the principal results of our study. But before this let us make precise the physical situation we are considering. The systems considered will be at mechanical equilibrium and subject to time-independent boundary conditions. Moreover they will be open and far from thermodynamic equilibrium.

We consider a chemical system in which A transforms into B through intermediates X , Y ,...



If the system is left alone it reaches the state of thermodynamic equilibrium. The affinity of the system is then zero. We now continuously add A and remove B in such a way that the resulting affinity would correspond to a situation far from thermodynamic equilibrium, i.e. : $|$ affinity $/RT|>1$.

It is also assumed that the kinetics equations are non linear

$$\frac{dX}{dt} = F(X,Y,\ldots)$$

F being some non linear function of X , Y ....

We have shown that these systems have the following properties

a) Systems close to thermodynamic equilibrium always evolve towards a disordered regime. Which is asymptotically stable with respect to disturbances.

b) Structures may appear spontaneously in systems maintained beyond a critical distance from thermodynamic equilibrium and provided the kinetics is autocatalytic, or crosscatalytic.

c) Whenever a structure appears in a system described by b), the steady state obtained by the extrapolation of the close to equilibrium behaviour, the thermodynamic branch, becomes unstable. The system jumps to a new regime which may

correspond to a spatially or temporally organized state (5) (6), or a spatiotemporal organization in the form of concentration waves (7). Transitions between multiple steady states can also appear in homogeneous media. Such organized systems have been called dissipative structures (8), (9).

These general results have been confirmed both by the study of models and by laboratory experiments (10),(11).

In spite of the diversity of the situations which may arise in non-linear systems far from equilibrium, there is a general thermodynamic theory underlying these nonequilibrium order phenomena, which shows that when the system becomes instable, the excess entropy production changes sign. The additional amount of dissipation (entropy production) introduced by the internal fluctuations which are assumed to be small, becomes negative. In this way the stability properties are connected with thermodynamic functions of direct experimental interest.

Here we shall give in some detail only two examples of dissipative structures showing coherent behaviour far from equilibrium

Both exemples pertain to the functioning of biological membranes. These are the active transport in E. Coli due to induction of lac operon (12), and the problem of permeability of excitable membranes (13).

1) β-GALACTOSIDASE INDUCTION AND ACTIVE TRANSPORT

The investigations of Jacob and Monod (14) Novick and Weiner (2) have shown that at the dellular level, the induction of β-galactosidase in E. Coli is an " all-or-none" phenomenon.

Bacterial populations grown at low concentration of inducer in a fixed medium are composed of individuals which are in either of two possible steady states : maximally induced or non induced. The bacteria can be shifted from one stable

state to the other by transitory variations in the environment. The " all-or-none" character can be understood in terms of the functioning of " galactoside permease" (15),(16). This protein is an integral part of the transport system by which lactose is accumulated and utilised in the organism. Permease is a genetically controlled protein and is a product of the Y gene of the lac operon which also controls the synthesis of β -galactosidase itself. Thus lac permease is induced by its own product: the induction phenomenon is autocatalytic.

In what follows we incorporate the existing experimental data on kinetics of β -galactosidase induction into a simplified model.

The model

The lactose operon is one of the most studied problems in bacterial genetics. These is a wealth of experimental data on β-galactosidase induction kinetics (17). In all experiments the induction of the enzyme (by measuring its activity) is investigated while adding a non metabolisable inducer (gratuitous inducer) into the system. The observed experimental curves, for mutants producting permease are sharp sigmoidal curves showing a sudden jump in the quantity of the enzyme, while the inducer concentration changes relatively slowly. For permeasless stains the magnitude of the jump is much less and takes place for inducer concentrations of 10 orders of magnitude higher.

The process of permeation of the inducer through the bacterial membrane has also been widely investigated (11). It is shown that for permease mutants the transport of inducer is via active transport e.g: against a concentration gradient. It seems to us that the following simplified model describes the most important features of the induction processes.

$$R' \underset{k_2}{\overset{k_1}{\rightleftharpoons}} R$$

$$R + O^+ \underset{k_4}{\overset{k_3}{\rightleftharpoons}} O^-$$

$$R + I_i \underset{k_6}{\overset{k_5}{\rightleftharpoons}} F$$

$$\alpha + O^+ \xrightarrow{k_7} M + E + O^+$$

$$M + I \underset{k_9}{\overset{k_8}{\rightleftharpoons}} M + I_i$$

$$M \underset{k_{11}}{\overset{k_{10}}{\rightleftharpoons}} F'$$

$$E \underset{k_{11}}{\overset{k_{10}}{\rightleftharpoons}} F''$$

R and R' are the active and inactive (precursor) forms of repressor molecules (19). O^+ and O^- are respectively the probabilities that the regulatory gene be opened or closed (with the obvious conservation condition $O^+ + O^- = 1$). I_i is the concentration of "gratuitous" inducer inside the bacteria and I the concentration outside. E and M are respectively the enzyme and permease concentrations.

The first step describes the activation of repressor molecules (19). The second and third steps denote respectively operator-repressor and repressor-inducer complex formation (20). The next step is a short cut to describe protein synthesis on DNA templates.

The lactose permease system for transport of inducer is a much more involved and extensively studied mechanics (18). However it does not seem to contain relevant non linearities as far as the β-galactosidase induction process is concerned. We summarise the induced transport by the simple step (5). The asymmetry of binding of inducer by permease on the exterior as compared to the interior membrane has been thought to be the primary cause of the internal accumulation of the galactosides

(18), (17). To describe this fact we take $k_3 > k_5$. The last two steps account for the protein loss.

We write the kinetic equations of the scheme I assuming that the system is homogeneous. The steady states of the system have been studied numerically on a C.D.C. computer for a wide range of the values of the coefficients. A typical result is shown in Fig.1.

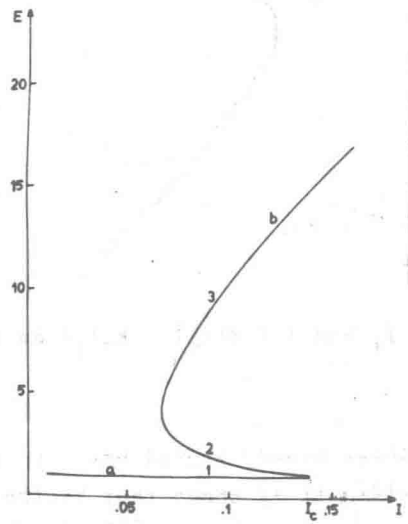


Fig.1. Multiple steady states of E versus inducer concentration. $k_1 = 1$; $k_2 = .01$; $k_3 = 10$; $k_4 = .01$; $k_5 = 5$; $k_6 = 1$; $k_7 = 1$; $k_8 = 5$; $k_9 = .1$; $k_{10} = .01$; $k_{11} = 1$; $\quad = 4$; $F = .1$; $F' = .01$; $F'' = .01$; $R' = .8$ (in proper units).

One begins with a very low concentration of inducer and gradually adds substrate into the system; the computed concentration of β-galactosidase is low. This corresponds to the uninduced bacteria and is shown by branch (a) of Fig.1. When a threshold concentration I_c is reached the concentration of enzyme jumps to branch (b). In this state the bacteria are induced.

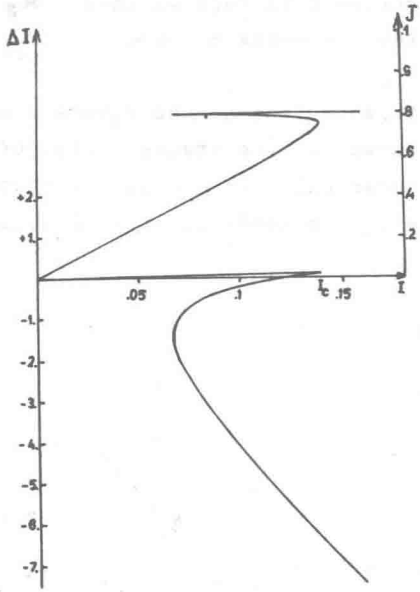


Fig. 2. $\Delta I = I - I_i$ and $J = M(k_8 I - k_9 I_i)$ as a funtion of I.

The stability of these steady states has been investigated by normal mode analysis. It is shown that states one and three are stable and two is unstable. In Fig. 2, we plot the flux of inducer through the bacterial membrane $J = M(k_8 I - k_9 I_i)$ and the gradient $\Delta I = I - I_i$ as a function of inducer concentration. It is seen that for uninduced bacteria before the cri ical point of transition the inducer entry is carrier mediated. ΔI and J have the same sign.

In the region of multiple steady states, where the bacteria are induced, there is a sudden jump of ΔI towards negative values while J remains positive. The change of sign of ΔI, shows that for these bacteria there is an uphill transport of inducer after the point of instability of branch (a) is reached. We have here an example of active transport in the particular case of the lactose transport system.

The over simplified steps (5) for the carrier transport of inducer across the membrane can be replaced by a much more realistic and elaborated model proposed by Kennedy and al. which one considers that the M protein (permease) has two conformational states with different affinities of binding for the substrate.

Moreover the permeation phenomenon is accomplished through energy consuming steps.

The study of this new model shows essentially the same type of behaviour as the simplified model described above.

Recent work (21) in the study of repressor-inducer interaction suggests that the repressor is an allosteric protein which in the absence of inducer combines with the operator and which in its presence assumes a form where this affinity is lost. The operator can again synthetise protein normally. Moreover the interaction between the repressor and inducer is quadratic (22). These additional features of operator-repressor interaction add new types of non-linearities to the system. Here they would merely amplify the distance separating multiple steady states[+)] but their role is prominent in the occurrence of logistic shaped kinetic curves for the induction in permeaseless strains.

3. PERMEABILITY OF EXCITABLE MEMBRANES

In connection with the phenomenon of nerve excitation, we shall study here the coupling between co-operative behaviour of units of a biological membrane and its non-equilibrium environment (23). This effect might be responsible for essential aspects of excitation phenomena and consequently play an important role in the transmission of information in living systems.

+) We verified this conjecture for the case $R + 2I_1 \rightleftharpoons F$

Let us make the following physical assumptions concerning the excitable membranes:

1) the membrane is considered as an isothermal lattice separating two bulk solutions of different electrochemical potential (asymmetric environment). The system is considered to be open and there is a flux of permeant across the membrane.

2) the lattice is formed by lipoproteic units or protomers. Each protomer has two specific binding sites for P , one on the inner face of the membrane, the other on its outer face.

The permeant therefore both binds and permeates across the membrane. Transport takes place by a "jump" of P from one class of sites to the other (24). The presence of specific binding sites for P on the protomer express the selectivity of excitable biological membranes for substrates. Moreover the distinction between inner and outer sites of the membrane is introduced to take into account the diversity of the experimental data on membranes.

3) The protomers can exists reversibly in two different conformations S or R. The affinity and the permeability of P are altered when a transition takes the protomers from one conformation to another.

4) Cooperative interactions are established between protomers within the membrane lattice through a conformational coupling.(25).

5) We consider the existence of an equilibration layer on both sides of the membrane. These layers have properties different from the bulk of the solution. The concentration of the permeant in this region is determined by diffusion across the membrane and the diffusion between the bulk of the solution and this equilibration layer.