

## On Observable Discontinuities and Coherence in the Kinetics of Enzymically Reacting Systems

J. A. CHRISTIANSEN

*Sundvænget 9, Hellerup, Denmark*

It is shown that there are thermodynamic arguments for the belief, that the cyclic transformations which the individual molecules of an enzyme in a macroscopic reacting system are forced to run through during their action as catalysts are in phase, are coherent. A necessary consequence of this behaviour is, that the reaction proceeds in observable jumps, whose magnitude is determined by the amount of enzyme present in the system. Experimental evidence, which seems to support this view is quoted.

This paper contains an extension of considerations from former papers of the author<sup>1-3</sup>.

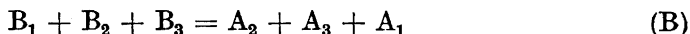
In most mechanical or even quantum-mechanical pictures of an elementary chemical reaction the process is considered to be a continuous one. The picture may be more or less refined, but the fundamental assumption is always the one due to Arrhenius, that a molecule or a complex of molecules to react must pass a state in which its energy is maximum.

Arrhenius<sup>4</sup> called this state the active one. Later on R. Marcelin<sup>5</sup> denoted it as the critical state, while J. N. Brønsted<sup>6</sup> in a paper in German used the name "Übergangszustand". The corresponding name in English "transition state" was used by M. Polanyi and M. G. Evans<sup>7</sup>, and this name has since been used exclusively by H. Eyring and his school.

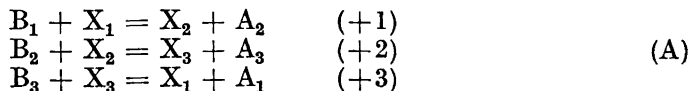
But even if the single elementary reaction be described as proceeding continuously, it is evident that statistically an elementary reaction must be considered as practically speaking discontinuous. This is so, because the life times of the reactants and the resultants are immensely much larger than that of the intermediate complex. Therefore we commit only a very small error if we state that a molecule or a complex of molecules is either in the reactant state or in the resultant state. Or we may say, that the reaction takes place in a jump from one state to the other through the transition state. However, in a macroscopic system, containing an immense number of reacting molecules

the jumps are randomly distributed in time, and the discontinuity is therefore not observable. But this statement may not be true in the case where a reaction is catalyzed, *e.g.* by an enzyme.

Let a reaction, which has a positive affinity from the left to the right



be catalyzed by an enzyme in three elementary steps:



$X_1$ ,  $X_2$ , and  $X_3$  being three different forms or compounds with the substrates ( $B_i$ ,  $A_i$ ) of the enzyme.

The sequence (A) means, that the reaction (B) forces the enzyme to undergo a cyclic transformation in the order 1231. What we observe is the occurrence or the disappearance of one species of the resultants or the reactants respectively. To fix our ideas let us assume, that we observe the occurrence of  $A_1$  which may be, *e.g.*, a (hydrated) hydrogen ion.

The liberation of this takes place in the moment when  $X_3$  is transformed into  $X_1$  which, according to the foregoing, is a sudden event, so long as only one molecule of the enzyme is considered. Or in other words, we may state that the enzyme molecule must be at a certain time in one of its three possible states.

Consider now the realistic case, where there are many molecules of the enzyme within a certain volume element. May we then assume that the different molecules are simultaneously in the same state or may we not? Or expressed in another way, is the instantaneous distribution of the enzyme on its different states the same everywhere in the reacting system?

According to thermodynamics it seems, that the answer to the latter question must be yes, because we know that, to change the state of the whole system from one in which the distribution is the same everywhere to one in which it is not, requires work. Therefore the former state of the system is more probable than the latter and, as usual in statistical thermodynamics, so much more so that it is, practically speaking, the only possibility. We may therefore say that the cyclic transformations of the individual enzyme molecules must always be in phase or, that the system is coherent in respect of transformations of the enzyme.

This coherence would have an important consequence: As mentioned above the transformation of an  $X_3$  into an  $X_1$  is accompanied by a liberation of  $A_1$ . If now coherence exists this means, that all the enzyme molecules  $X_3$  react simultaneously that is, an amount of  $A_1$  which is stoichiometrically equivalent to the amount of enzyme in the system is suddenly released. If, therefore, the liberation of  $A_1$  in our system is observed and taken as a measure of the extent of the overall reaction (B) it is evident, that we observe a discontinuous progress of the reaction. Or, in other words, the reaction is observed to proceed in consecutive jumps, each representing the same number of individual reactions according to (B). It must be added, however, that the author is not

able at present to indicate a mechanism, by means of which the coherence is maintained. It is evidently a question of great importance whether or not the peculiar behaviour described above of a reaction catalyzed by an enzyme has ever been found, and the answer seems to be in the affirmative: It is an experimental fact<sup>8</sup>, that certain (by no means all) nerves in their endings spontaneously generate electrical impulses which are transmitted through the nerve and which can be observed oscillographically by means of electrodes connected to the nerve in a suitable way. The signals as we may call them appear periodically, the period, which is often of the order of 0.01 to 0.1 s, being constant so long as the corresponding sense organ is not stimulated. Furthermore, and this is what mostly interests us here, the duration of the signal is only a small fraction of the distance in time between two consecutive signals. We thus get the impression, that periodically an outburst of "something" takes place which causes the electrical disturbance. Evidently this phenomenon might be understood on the lines described above if an enzyme catalyzing a chemical (metabolic?) reaction were located in the nerve ending, the substrates being supplied from its surroundings. This again would be a corroboration of the widely accepted assumption, that everything which happens in a living organism is caused by chemical reactions. It may be added that it is a fact that chemical reactions go on in nerves as in other cells of the living organism.

Besides this to a certain extent direct evidence for the existence of discontinuities in the course of an enzymic reaction, evidence of a more indirect nature seems to exist: In most (not in all) cases of enzymic reactions it can be shown that steady state is arrived at in the course of an exceedingly short time. In such cases a function  $\varphi$  of the extent of reaction  $\xi$  can always be found, which obeys the equation

$$t = \varphi(\xi)$$

where  $t$  is the time at which the extent of reaction has reached the value  $\xi$ . If now, as hitherto assumed,  $\xi$  increases continuously with time, it must be possible to determine the rate of the reaction with an accuracy which is only limited by the inaccuracy of the experiments. If, however, the reaction proceeds in observable jumps it may well happen, that the accuracy obtained is less than that estimated from the properties of the experimental procedure. We have had in the institute of physical chemistry of the university of Copenhagen indications of such a situation. However, as we might have overestimated the accuracy of our measurements, the results were not published. Then a Russian colleague, S. E. Shnoll, referring to the present authors paper<sup>2</sup>, sent him a paper in Russian<sup>9</sup>, in whose English summary Shnoll explicitly states, that his determinations of the rate of a certain reaction show deviations, which certainly cannot be explained by inaccuracies in the experimental procedure.

The different pieces of evidence mentioned above may not seem sufficiently convincing when they are taken separately. But when taken together they seem to point out that reactions of the types mentioned show discontinuities which are macroscopically observable. Or as we may say, the single cyclic transformations in the macroscopic system are in phase, are coherent.

## REFERENCES

1. Christiansen, J. A. *Acta Chem. Scand.* **11** (1957) 1436.
2. Christiansen, J. A. *Z. Elektrochem.* **62** (1958) 225.
3. Christiansen, J. A. *Acta Chem. Scand.* **12** (1958) 352.
4. Arrhenius, S. *Z. physik. Chem.* **4** (1889) 226.
5. Marcelin, R. *Ann. phys.* **3** [9] (1915) 120.
6. Brønsted, J. N. *Z. physik. Chem.* **102** (1922) 169.
7. Polanyi, M. and Evans, M. G. *Trans. Faraday Soc.* **31** (1935) 875.
8. *Cf. Ref.*<sup>1</sup>, p. 225.
9. Shnoll, S. E. *Voprosy Med. Khim.* **4** (1958) 443.

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