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P. M. Du Bois

Geological Survey of Canada,
Ottawa.

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BIOCHEMISTRY

Kinetic Study of Dextransucrase based on the Langmuir Adsorption Isotherm

The action of the polysaccharide-synthesizing enzyme dextransucrase has been described previously in terms of *p*H [1] and the effect of various alternate receptors[2,3]. We have now examined the dependence of rate of reaction on the concentration of enzyme. The results are interpreted on the basis of adsorption kinetics[4], where the substrate (sucrose) and acceptor are adsorbed simultaneously on adjacent sites of the enzyme. This is followed by glucosyl transfer from sucrose to acceptor. No reaction occurs between bound sucrose and acceptor in solution, and there is no separate hydrolytic step prior to the transfer of the glucosyl group.

The kinetic studies were carried out by exposing sucrose solutions to varying amounts of crude enzyme solution at 25° C. and *p*H 5·2, and measuring the amount of fructose produced at a given time. The enzyme was prepared as reported elsewhere[1]. Sucrose solution (0·2 ml. of a 0·01 *M* solution in 0·05 *M* acetate buffer *p*H 5·2) was incubated with 0·02–1·6 ml. of the enzyme solution (assaying 49 units/ml.) and buffered to make a final volume of 1·8 ml. After ½ hr. the reaction was quenched with 0·1 *M* sodium hydroxide and the reducing sugars determined colorimetrically[5,6]. A plot of rate against enzyme concentration is shown in Fig. 1.

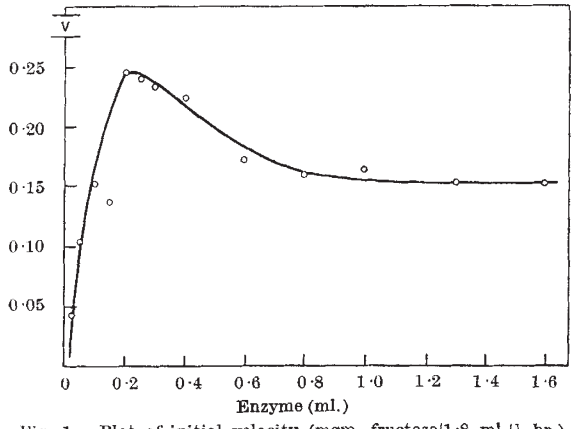

Fig. 1. Plot of initial velocity (mgm. fructose/1·8 ml./½ hr.) against enzyme concentration

The appearance of a maximum in such a plot is rare; however, it has been reported for the lactic dehydrogenase system[7]. Laidler and Socquet[4] have treated this case theoretically and have derived the following rate equation:

where k is the rate constant for the decomposition of the complex; S_1 and S_2 are the two substrates, in this case sucrose and acceptor; K_1 and K_2 are the corresponding equilibrium constants for the formation of the binary complex; K_1' and K_2' are the equilibrium constants for the cross adsorption of S_2 on site 1 and S_1 on site 2 respectively. It is seen that equation (1) predicts linearity of rate against enzyme or sucrose at low concentration, but an inverse relationship and hence a maximum at high concentration.

At high enzyme concentration, the substrates are spread out over a large number of enzyme sites. The observed decrease in rate is attributed to the decreased probability that adjacent sites will be occupied in these circumstances. The previously observed maximum in the rate against sucrose concentration[8] indicates that sucrose is cross-adsorbed on the acceptor sites (which may well be identical with the donor sites) and thus inhibits the reaction. Attempts to observe maxima in S_2 by adding alternate acceptor have been complicated by the presence of the natural acceptor. Thus far, only a roughly linear increase of rate with acceptor concentration has been observed.

If a reaction were occurring between bound substrate and unbound acceptor, no maximum would occur. Furthermore, it has been shown[4] that in cases where one substrate is solvent there is no maximum in the enzyme curve. Hence the possibility of a hydrolytic reaction with sucrose followed by a rate-determining combination with dextran is eliminated. The presence of the observed maxima thus fits only the case of simultaneous two-substrate adsorption.

W. Brock Neely
C. F. Thompson

Biochemical Research Laboratories,
The Dow Chemical Co.,
Midland, Michigan.

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Agar Electrophoresis of Normal Soluble Proteins in Guinea Pig Liver

Hitherto, neither free electrophoresis[1] nor electrophoresis on filter paper of the soluble proteins in liver (ref. 2, and Smetana, R., and Kořínek, J., personal communication) has afforded satisfactory delimitation of a sufficient number of fractions. After obtaining a number of unsatisfactory results on filter paper, we turned our attention to electrophoresis on agar gel.

Healthy guinea pigs weighing between 300 and 900 gm. were lightly anæsthetized with ether before being perfused with normal saline. The liver was ground in a mortar with quartz sand. 1–3 ml. of a 0·7 per cent solution of sodium chloride (the amount depending on the size of the liver) was added and the whole stored in a refrigerator. 24 hr. later it was centrifuged at 6,000 r.m.p. for 2–3 hr.

$$v = \frac{kK_1K_2[E]_0[S_1]_0[S_2]_0}{\{1+[E]_0(K_1+K_1')+K_1[S_1]_0+K_1'[S_2]_0\}\{1+[E]_0(K_2+K_2')+K_2'[S_1]_0+K_2[S_2]_0\}} \quad (1)$$