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ENZYME REACTIONS IN DENTAL PLAQUE MATRIX
MODIFICATION OF VELOCITY BY THE PRESENCE OF POLYMERS

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The study was undertaken to evaluate the influence of inert polymers on the reactions of extracellular plaque enzymes. It was demonstrated that several polymers, including dextran, caused a marked increase of the reaction rates of *levan sucrase* as well as *levan hydrolase* from *Streptococcus salivarius* and *Strep. mutans*, respectively. In the case of levan hydrolase it was furthermore observed that the relatively low diffusion rate of levan limited the collision frequency between enzyme and substrate, leading to an initially decreased reaction rate. This effect was dependent on the levan concentration, and it was enhanced by dextran. The findings are discussed in the light of known physico-chemical phenomena, and the implications for enzymes within the dental plaque matrix are noted.

It is known that dental plaque consists of bacteria embedded in an intermicrobial matrix. The plaque matrix contains extracellular enzymes of bacterial origin, among which levan sucrase and levan hydrolase are notable examples. Both enzymes have been demonstrated in dental plaque (*Manly & Richardson*, 1968), and they are known to be formed by oral streptococci (*Guggenheim & Schroeder*, 1967; *da Costa & Gibbons*, 1968; *Carlsson*, 1970; *Kelstrup & Gibbons*, 1970).

In addition to water and diffusible compounds (amino acids, carbohydrates, inorganic salts, etc.) high molecular weight polysaccharides are a constituent of the plaque matrix. The predominant polysaccharides are glucans (*Wood*, 1969; *Hotz, et al.*, 1972) which seem to be relatively resistant to degradation by the bacteria of plaque (*Gibbons & Banghart*, 1967). It is therefore obvious that the extracellular enzymes within dental plaque act in the presence of glucans, and consequently the purpose of the present

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investigation was to study the influence of polymers on the reaction rate of the selected enzymes, levan sucrase and levan hydrolase.

MATERIALS AND METHODS

Enzyme preparations. Levan sucrase (β-2,6-fructan: D-glucose 6-fructosyltransferase, EC 2.4.1.10) was synthesized by *Streptococcus salivarius* strain 1A (*Kelstrup & Gibbons*, 1970). For enzyme synthesis bacteria were grown for 24 h in 5 % CO_2 and 95 % N_2 at 35°C in a dialyzed Trypticase medium supplemented with 0.2 % glucose (*Kelstrup & Funder-Nielsen*, 1972). The extracellular levan sucrase formed by *Strep. salivarius* strain 1A is capable of synthesizing levan from sucrose, but not from various other carbohydrates tested (*Kelstrup & Gibbons*, 1970). Similar enzymes have been studied extensively in other bacteria (for review, see *Carlsson*, 1970) and have been shown to transfer the β-fructofuranosyl group of sucrose to suitable acceptors. The products of the enzymes are glucose, fructose, oligosaccharides, and levan. For practical purposes the liberation of glucose may be taken to indicate the velocity of the enzyme reaction.

Extracellular levan hydrolyzing enzymes are elaborated by *Streptococcus mutans* and *Strep. salivarius* (*da Costa & Gibbons*, 1968; *Kelstrup & Gibbons*, 1970). For this study levan hydrolase was synthesized by *Strep. mutans* strain C67—1 (*de Stoppelaar, et al.*, 1971). The bacteria were grown for 3 days under the above mentioned conditions in the Trypticase basal medium, supplemented with 0.05 % glucose and 0.15 % levan, synthesized by *Strep. salivarius* strain 1A (*Kelstrup & Gibbons*, 1970). Residual levan could not be detected by ethanol precipitation of the culture supernate.

Dialyzed cell-free culture supernate served as source of extracellular enzymes, as described earlier (*Kelstrup & Funder-Nielsen*, 1972). It might be stored frozen for several weeks without appreciable loss of activity. The pH-optimum of both enzyme preparations was at or close to pH 6.0. Levan sucrase was present in glucose-grown cultures. Levan hydrolase was inducible by levan; small amounts of enzyme were present in glucose-grown cultures, but the quantity was increased more than ten-fold when levan was included in the growth medium. This preparation was capable of degrading inulin, indicating that it was active against β-2,1 (in addition to β-2,6)-fructofuranosyl linkages, similarly to that studied by *da Costa & Gibbons* (1968), but the activity was only 1/5 to 1/3 of that against levan.

Enzyme reaction analysis. Levan sucrase was assayed at 35°C in reaction mixtures of 0.5 ml sucrose at various concentrations in 0.2 mol/l Sørensen's

phosphate buffer (pH 6.0), 0.5 ml water or polymer solution, and 1.0 ml enzyme. All components were brought to the desired temperature and the reactions were started by the addition of enzyme. At various time intervals the reactions were stopped by addition of 1.0 ml 0.5 mol/l NaOH. The mixtures were deproteinized with NaOH-$ZnSO_4$ (*Fales, Russell & Fain,* 1967), and the amount of glucose liberated was determined enzymatically with glucose oxidase (»Glox novum», Kabi AB, Stockholm). »Glox novum» contained invertase, which was inactivated by the addition of 10^{-4} mol/l $AgNO_3$; a precipitate of AgCl was removed by centrifugation. When a polymer was used it was included in the glucose standard. The initial enzyme velocity was estimated and expressed as μg glucose liberated per ml per min. under the standard conditions.

Levan hydrolase was assayed at 35°C in mixtures of 0.5 ml levan (the levan was formed by *Strep. salivarius* strain 1A (*Kelstrup & Gibbons*, 1970) in 0.2 mol/l phosphate buffer (pH 6.0), 0.5 ml water or polymer solution, and 1.0 ml enzyme. The reactions were carried out as the levan sucrase reactions, and after deproteinization the amount of reducing sugar liberated per ml was determined (*Somogyi*, 1945) with fructose as standard.

K_m and V were estimated by means of Lineweaver-Burk plots of the data (*Lineweaver & Burk*, 1934). All regression lines were calculated by the least square method.

Chemicals. »Glox novum» was from Kabi AB, Stockholm, Sweden. Dextran T 40 (lot. no. 2514) with an average molecular weight of approx. 40,000 was obtained from Pharmacia, Uppsala, Sweden, and polyethylene glycol (PEG 4,000) with a molecular weight of 3,000 to 3,700 was from Koch-Licht, Colnbrook, England. Other chemicals were of analytical grade.

RESULTS

Levan sucrase. It was evident from the Lineweaver-Burk plots that the presence of polymers changed the reaction rate appreciably (Fig. 1). The apparent K_m was 0.017 mol/l in buffer, but only 0.008 mol/l in the presence of 75 mg/ml dextran, and V appeared to be increased. To demonstrate that not only dextran, but also a synthetic polymer would influence the reaction rate, polyethylene glycol was included in the reaction mixtures at a concentration of 20 mg/ml. The same effect as with dextran was seen, the apparent K_m being lowered to 0.014 mol/l and V being increased.

Levan hydrolase. It was found that the presence of dextran affected the enzyme activity in a rather complex manner. A representative example is

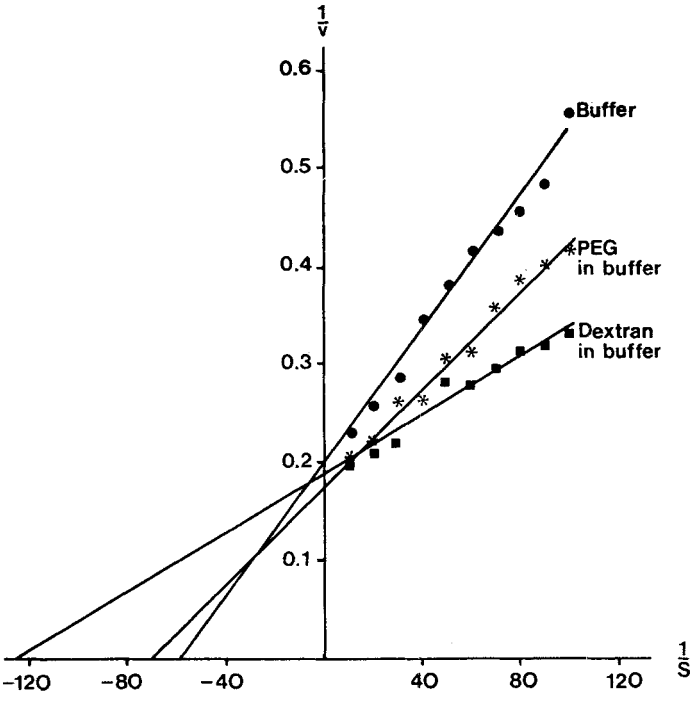

Fig. 1. Lineweaver-Burk plots of data obtained with levan sucrase in buffer, buffer containing PEG (20 mg/ml), and buffer containing dextran (75 mg/ml). The substrate concentration (S) is expressed as mol/l, the initial reaction velocity (v) as release of glucose in μg/ml/min.

shown in Fig. 2. It is seen that the rate of release of reducing sugar in reaction mixtures containing dextran (conc. 75 mg/ml) was initially decreased, while later it was enhanced, compared to similar mixtures without dextran. Furthermore, the initial decrease in enzyme activity appeared to depend on the substrate concentration, being most pronounced at low levan concentrations. It was observed in buffer as well as in dextran solutions, but it appeared to be augmented by the presence of dextran (Fig. 3).

DISCUSSION

Enhanced chemical potential by exclusion of macromolecules. *Laurent* (1968) in a review showed that in a model system of spherical and rodshaped macromolecules in solution, the exclusion of the spheres from the solution would increase with decreasing radius or increasing asymmetry of the rods.

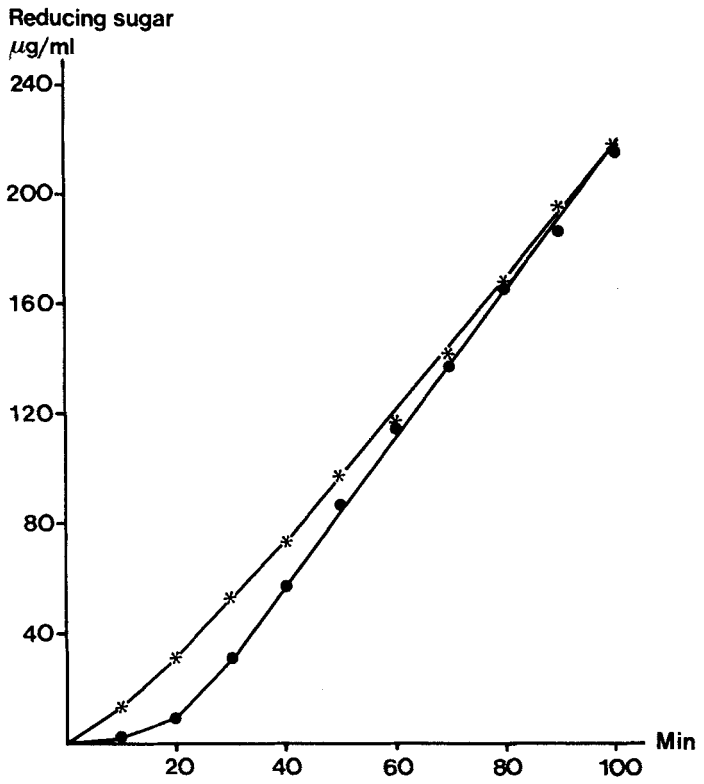

Fig. 2. Release of reducing sugar from levan (0.5 mg/ml) by levan hydrolase in buffer (✱——✱), and in buffer containing dextran (75 mg/ml) (●——●).

The available fraction of the total volume, which is inversely related to the excluded volume, is determined by the concentration of the rods and the radius of the spheres. As a consequence of the exclusion of macromolecules from part of the solution the chemical potential of the components of a mixture of macromolecules is often higher than when the same components are present individually, as noted also by *Giddings* (1970).

This model may be assumed to apply to dental plaque matrix, the »spheres» being extracellular anzymes and the »rods» being polysaccharides, and to the *in vitro* situation of the present study. *Laurent* (1971) predicted that the chemical activity of enzymes in a polymer solution should be increased, and this was actually found in experiments with several enzymes. The present study demonstrated a similar effect of polymers on two typical plaque matrix enzymes, levan sucrase and levan hydrolase.

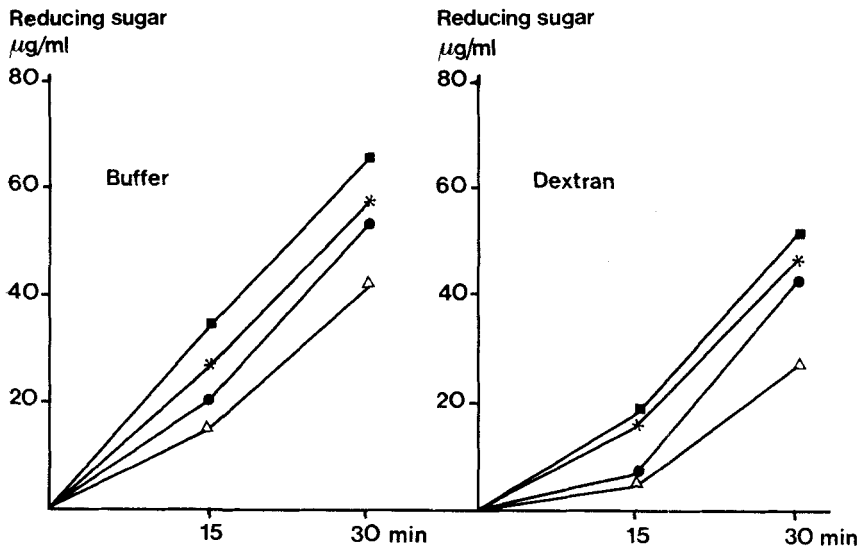

Fig. 3. Release of reducing sugar from various concentrations of levan by levan hydrolase in buffer, and in buffer containing dextran (75 mg/ml). Levan concentrations: 1.00 mg/ml (■——■), 0.50 mg/ml (✱——✱), 0.25 mg/ml (●——●), and 0.10 mg/ml (△——△)

Decreased diffusion coefficients. In addition to an enhancing effect on the chemical potential of macromolecules, polymers cause a decrease of the diffusion rates of all components present (for review, see *Laurent*, 1966). In enzyme reaction mixtures this leads to a decrease in the collision frequency between enzyme, substrate, and possible cofactors, and since the magnitude of this effect depends on the size of the molecules, it would be expected to be most pronounced with a high molecular weight substrate. This may explain the initially slow reaction rate of levan hydrolase, levan being a large molecule (*Avigad*, 1965). It is evident that the presence of dextran prolongs the »lag period» (Fig. 2, 3), and that it is also enhanced by lowering the levan concentration (Fig. 3), which would decrease the collision frequency. The ultimately increased reaction rate (Fig. 2) may then be ascribed to the exclusion effect described above.

Modification of enzyme reactions in plaque matrix. Thus, it may be concluded from theoretical considerations that the presence of polymers may have two effects on an enzyme reaction (1) an increase in reaction rate, due to an enhanced chemical potential of the enzyme, and (2) a decrease in reaction rate, because of a general decrease of diffusion. Both effects have been verified experimentally, by *Laurent* (1971) and in the present study.

It may, furthermore, be realized that under some circumstances the two effects may be superimposed, as found with the levan hydrolase reactions.

This makes the influence of polysaccharides in enzyme reactions in the plaque matrix difficult, or impossible, to predict. The concentration and molecular species of polysaccharides differs from area to area in the plaque matrix (*Saxton*, 1969), as do the bacteria (*Schroeder & Hirzel*, 1969) and presumably the extracellular enzymes they synthesize. It may, however, safely be assumed that the phenomena described in this communication are indeed occurring in dental plaque, modifying the enzyme reactions in the plaque matrix, and knowledge of the molecular size of the reactants may give some indication of the changes in reaction rate to be expected.

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