pH dependent effects of sodium ions on dextransucrase activity in *Streptococcus mutans*
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**Article Info**

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**Abstract**

Dextran sucrase (E.C 2.4.1.5) is a key enzyme in *S. mutans* for the metabolism of sucrose which helps in the adherence and accumulation of bacteria on tooth surface leading to the formation of dental caries. Dextran sucrase resembles in its catalytic properties with the brush border sucrase and exhibits pH dependent inhibitory and stimulatory effects in response to Na⁺. In this communication we studied the effect of monovalent cations on the activity of dextran sucrase from *S. mutans*. The percentage inhibition of dextran sucrase was 65% at 0.5 mM NaCl which enhanced to 90% at 20 mM sodium concentration. However there was no effect on dextran sucrase activity in presence of other monovalent cations (Rb⁺, Cs⁺, and K⁺) tested. Enzyme activity was enhanced 20–24% in acidic pH but was strongly inhibited (59–89%) around neutral and alkaline pH by 0.5–2.0 mM sodium chloride. Upon dialysis, 86% of enzyme activity was restored to control values. There was no effect of 2 mM NaCl on glucosyltransferase activity of the enzyme. Kinetic studies revealed that enzyme showed biphasic effects in response to Na⁺ ions. At acidic pH the enzyme exhibited mixed type of activation affecting both Vmax and Km, while in alkaline pH, the enzyme showed V- type effect reducing Vmax by 74% without affecting Km. The effects of sodium ions on dextran sucrase activity were specific, thus it can be useful to block its catalytic activity, and reducing the cariogenic potential of *S. mutans*.

1. Introduction

*Streptococcus mutans* is a gram positive organism which has been implicated in the pathogenesis of dental caries in humans [1]. The organism produces dextran sucrase (E.C.2.4.1.5.) which hydrolyzes sucrose a primary source used by the organism for its growth and adhesion to tooth surface [2]. Dextran sucrase exhibits both hydrolytic and glucosyltransferase activities and produces exopolysaccharides such as the dextrans which aid in the adhesion of microorganism onto the tooth surface thus causing the disease [3]. Similar to *S. mutans* dextransucrase the mammalian intestine contains brush border sucrase having both hydrolytic and glucosyltransferase activities [4]. One of the characteristic feature of the brush border sucrase is its activation by sodium ions [5]. The sodium stimulation of the enzyme is pH dependent and follows a non-compulsory kinetic mechanism resulting in V-type (change in Vmax) or K-type (change in Km) or mixed type enzyme kinetics [5]. Similar studies with regard to effect of sodium ions on dextran sucrase activities have not been reported. Thus the present investigation was undertaken to study the effect of monovalent cations on dextran sucrase activities in *S. mutans*. These findings indicated that sodium ions exhibit a biphasic effect on dextran sucrase activity which are also pH dependent. The enzyme activity is stimulated in the acidic pH around 5.2 whereas it is a strong inhibitor of enzyme activity in neutral and alkaline pH. The inhibitory effects are specific to sodium ions, since other monovalent cations tested were essentially inert.

2. Materials and methods

2.1. Reagents and buffers

All the chemicals used in the study were of analytical grade. Agar, Brain Heart Infusion Broth (BHI), exogenous dextrose, glucose, sucrose, peptone and maleic acid, sodium chloride, yeast, and ethanol were obtained from HiMedia Pvt. Ltd. (Mumbai, India). All other chemical used were obtained from E. Merck Pvt. Ltd. (Mumbai, India) or Sisco Research Lab Pvt. Ltd. (Mumbai, India). Universal buffer of 20 mM of various pH containing lithium hydroxide were prepared as described by Alvarado (1979) [6].

2.2. Bacterial strain and growth media

A lyophilized culture of *Streptococcus mutans* (MTCC 890) was...
obtained from microbial type culture collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. For revival of the bacteria, the strain was inoculated into growth medium-87 (1% dextrose, peptone, yeast extract, 0.5% BHI and 2% agar, pH 7.2–7.5) and the inoculated broth was incubated for 18–24 h at 37 °C. Culture purity was checked regularly by microscopic examination.

2.3. Enzyme preparation

Dextranucrase activity from S. mutans was purified using the modified method described previously [7]. The culture supernatant was treated with 55% (w/v) ammonium sulphate. The precipitate was collected by centrifugation at 12,000 g for 30 min and dissolved in 10 mM sodium maleate buffer (pH-6.8) containing 1 μM APMSF and 0.1 mM PMSF which was further subjected to 33% PEG-400 precipitation. The enzyme preparation was dialyzed against 5 mM lithium maleate buffer pH 6.8 using Dialysis Membrane-135 (HiMedia Pvt. Ltd. Mumbai, India). The dialysate was analysed for enzyme activity and protein concentration. Except otherwise stated, all procedures were carried out at 4 °C.

2.4. Determination of dextranucrase activity

Dextranucrase activity was assayed using standard reaction mixture containing 20 mM lithium maleate buffer (pH 6.8), 0.1 M sucrose, and enzyme in total volume of 0.5 ml was used to determine enzyme activity. After incubation for 30 min at 37 °C, the samples were assayed for glucose using Glucostat Kit (Reckon Diagnostic Pvt. Ltd.). Product formation was linear in the 30 min incubation period under the assay conditions. The enzyme activity was expressed as units/mg protein.

2.5. Effect of monovalent ions on dextranucrase activity

Effect of different concentrations (5–20 mM) of monovalent ions (Na+, Rb+, Cs+, and K+) from 0.5 mM-20 mM was studied by assaying the enzyme activity in lithium maleate buffer pH 6.8 in presence of 20 mM of sucrose as previously described [5]. The effect of calcium ions with concentrations from 0.5-20 mM on dextranucrase activity was also studied in presence of 20 mM sucrose. All the metal ions used were in the chloride form.

2.6. Effect of pH on the interactions of Na+ with dextranucrase

The effect of pH on dextranucrase interactions with Na+ was studied by varying the pH of the buffer from 3.6–8.0 and NaCl concentration of 2 mM. Enzyme activity was determined as described above.

2.7. Kinetics of Na+ ion effect on dextranucrase activity

Effect of different substrate concentrations on the inhibitory effect of Na+ on the dextranucrase activity was measured by evaluating the enzyme activity at 2, 4, 8, 12, 16, 20 and 24 mM of sucrose at pH 5.2, 6.8 and 8.0 in presence and absence of 2 mM Na+. Kinetic parameters Vmax, Km were calculated from reciprocal plot of Michaelis-Menten equation using programmable calculator [8]. The value of correlation coefficient ranged from 0.97 to 0.98 for various straight lines.

2.8. Effect of Na+ on the glucosyltransferase activity

Transferase activity of dextranucrase was assayed by a modified method as described by Mukasa et al. [9]. An adequate preparation of enzyme solution containing 0.1 M phosphate buffer (pH6.5), 41.7 mM sucrose, 34.3 μM dextran and 0.02% sodium azide was incubated for 15 h at 37 °C. The mixture was centrifuged at 17,000 g for 15 min to collect water insoluble glucan formed. The water soluble glucan was precipitated with 75% ethanol and collected by centrifugation at 17,000 g. The glucans formation was determined by phenol sulphuric acid method [10] where glucose is dehydrated to hydroxyl methyl furfural which forms a yellow brown coloured product with phenol with an absorption maxima at 490 nm.

2.9. Reversibility of Na+ inhibition

To determine whether the inhibition of dextranucrase by Na+ was reversible or not. The enzyme preparation containing 2 mM NaCl was dialysed extensively against 5 mM lithium maleate buffer pH 6.8 for 20 h at 4 °C and the control without NaCl was also run under these conditions. The enzyme activity was measured before and after dialysis of enzyme preparation.

2.10. Statistical analysis

The results were analysed by GraphPad Prism (version 5.01) and the data was expressed as mean ± S. D except otherwise stated. The acceptable level of significance was P < 0.05 for each analysis.

3. Results

3.1. Effect of Na+ ions on the activity of dextranucrase activity in S. mutans

Activity of dextranucrase was studied in 20 mM lithium maleate buffer pH 6.8 at different concentrations of sodium ions in its chloride form ranging from 0.5 mM to 20 mM. As shown in Fig. 1, there was a marked decrease in the activity of dextranucrase by increasing NaCl concentration. At 0.5 mM concentration the percentage enzyme inhibition was 65%. Which was increased to 90% at 2 mM NaCl concentration. Thereupon up to 20 mM there was little change in enzyme activity yielding, 95% decline in enzyme activity compared to the control.

3.2. Effect of monovalent cations and Calcium ions on dextranucrase activity

Taking 20 mM lithium maleate buffer pH 6.8 as the base medium since Lithium is considered to be inert [11] the effect of various cations...
in their chloride form of Rb\textsuperscript{+}, Cs\textsuperscript{+}, K\textsuperscript{+} and Ca\textsuperscript{2+} from 0.5-20 mM on the activity of dextransucrase was also studied. There was essentially no effect on the enzyme activity in presence of Rb\textsuperscript{+}, Cs\textsuperscript{+}, and K\textsuperscript{+}. However as reported earlier by Mohan and Goyal [3] dextransucrase activity was stimulated in presence of Ca\textsuperscript{2+} (0.5–20 mM). Increasing concentration of NaCl in the assay mixture progressively reduced dextransucrase activity, and at 20mM NaCl, the enzyme activity was reduced by nearly 90% (Table 1).

### 3.3. Effect of pH on dextransucrase activity in the presence and absence of Na\textsuperscript{+} ions

The effect of 2 mM Na\textsuperscript{+} on dextransucrase activity was studied in sodium free medium at different pH ranging from 5.0-8.0. The data was plotted as Va/Vo vs pH, where Va is the enzyme activity in presence of 2 mM NaCl and Vo represent enzyme activity in absence of NaCl. At the acidic pH, presence of Na\textsuperscript{+} enhanced the activity of dextransucrase by 20–24% compared to that in the absence of Na\textsuperscript{+}. However at alkaline pH (7.2–8.0) there was a marked decline (60–70%) in the activity of dextransucrase. These results indicated biphasic effect of Na\textsuperscript{+} on dextransucrase activity in S. mutans as a function of pH, an activator of the enzyme activity in the acidic pH but a powerful inhibitor at the alkaline pH (Fig. 2).

### 3.4. Effect of Na\textsuperscript{+} on the glucosyltransferase activity

Dextransucrase exhibits both hydrolytic and transferase activities. It catalyses the transfer of glucosyl residues from sucrose to dextran to form complex polysaccharides. Thus the effect of Na\textsuperscript{+} ions on transferase activity of dextransucrase was also examined in sodium free medium. There was essentially no effect of the metal ion on the transferase activity under the assay conditions as shown in Table 2.

### 3.5. Reversibility of Na\textsuperscript{+} inhibition

To examine the nature of interaction of Na\textsuperscript{+} with dextransucrase, the preparation containing 2 mM Na\textsuperscript{+} was dialysed. The enzyme activity was restored by 86% after dialysis compared to the control enzyme preparation. This suggested that dextransucrase inhibition by Na\textsuperscript{+} is essentially reversible in nature although the enzyme activity was still 14% low compared to the control (Table 3).

### 3.6. pH dependent effects of Na\textsuperscript{+} on the kinetics of dextransucrase

Effect of substrate concentration on the dextransucrase in the absence and presence of 2 mM Na\textsuperscript{+} was studied by assaying enzyme activity at pH 5.2, 6.8, 8.0 in sodium free medium. The data was analysed by Line-weaver-Burk plot to calculate the Km and Vmax of the enzyme.

### Table 1

| Conc. of cations | Activity (Units/mg protein) |
|------------------|-----------------------------|
| Sodium           |                             |
| 0.5 mM           | 0.14 ± 0.032                |
| 1 mM             | 0.08 ± 0.033                |
| 2 mM             | 0.04 ± 0.015                |
| 5 mM             | 0.04 ± 0.014                |
| 10 mM            | 0.03 ± 0.011                |
| 15 mM            | 0.02 ± 0.006                |
| 20 mM            | 0.019 ± 0.0019              |
| Rubidium         |                             |
| 0.5 mM           | 0.414 ± 0.029               |
| 1 mM             | 0.396 ± 0.007               |
| 2 mM             | 0.389 ± 0.004               |
| 5 mM             | 0.387 ± 0.005               |
| 10 mM            | 0.398 ± 0.011               |
| 15 mM            | 0.392 ± 0.009               |
| 20 mM            | 0.367 ± 0.025               |
| Calcium          |                             |
| 0.5 mM           | 0.202 ± 0.009               |
| 1 mM             | 0.256 ± 0.020               |
| 2 mM             | 0.287 ± 0.004               |
| 5 mM             | 0.389 ± 0.003               |
| 10 mM            | 0.507 ± 0.007               |
| 15 mM            | 0.561 ± 0.014               |
| 20 mM            | 0.6740 ± 001                |
| Cesium           |                             |
| 0.5 mM           | 0.321 ± 0.003               |
| 1 mM             | 0.326 ± 0.006               |
| 2 mM             | 0.325 ± 0.004               |
| 5 mM             | 0.313 ± 0.004               |
| 10 mM            | 0.316 ± 0.001               |
| 15 mM            | 0.303 ± 0.015               |
| 20 mM            | 0.303 ± 0.003               |
| Potassium        |                             |
| 0.5 mM           | 0.213 ± 0.0015              |
| 1 mM             | 0.207 ± 0.0037              |
| 2 mM             | 0.203 ± 0.0246              |
| 5 mM             | 0.212 ± 0.003               |
| 10 mM            | 0.199 ± 0.0158              |
| 15 mM            | 0.219 ± 0.0087              |
| 20 mM            | 0.229 ± 0.0464              |

Values are mean of three independent experiments (mean ± SD).
Metal ions were in their chloride form.
Enzyme activity as Units/mg protein.
Table 4
Effect of Na⁺ on the glucosyltransferase activity of dextranacruate at pH 6.8 in S. mutans.

The glucosyltransferase activity of dextranacruate in S. mutans was studied in presence of different concentrations of sodium ions (NaCl) at pH 6.8. Enzyme activity was calculated as Units/mg protein. Values are mean ± SD of three independent experiments.

| Na⁺ (mM) | Transferase activity (Units/mg protein) |
|---------|----------------------------------------|
| 0       | 1.265 ± 0.019                          |
| 0.5     | 1.273 ± 0.019                          |
| 1       | 1.242 ± 0.015                          |
| 2       | 1.241 ± 0.0255                         |
| 5       | 1.327 ± 0.090                          |
| 10      | 1.23 ± 0.106                           |
| 15      | 1.267 ± 0.107                          |
| 20      | 1.625 ± 0.099                          |

Values are mean ± SD of three independent experiments.

Table 3
Reversibility of inhibition by Na⁺ ions on dextranacruate in S. mutans.

The reversible nature of sodium inhibition on dextranacruate in S. mutans was carried out by dialysing the enzyme containing 2 mM NaCl overnight. The enzyme activity of both control and test samples was measured before and after dialysis. Enzyme activity was calculated as Units/mg protein. Values are mean ± SD of three independent experiments.

| Assay system | Activity (Units/mg protein) | % inhibition |
|--------------|----------------------------|--------------|
| Control (undialysed) | 0.203 ± 0.0085 | -            |
| Test (undialysed + 2 mM Na⁺) | 0.0084 ± 0.0024 | 96           |
| Control (dialysed) | 0.0936 ± 0.0078 | -            |
| Test (dialysed + 2 mM Na⁺) | 0.0803 ± 0.0205 | 14           |

Values are mean of three independent experiments (mean ± SD). ***p < 0.0001.

Table 2
Kinetic parameters of dextranacruate Km, Vmax at different pH in the presence of 2 mM Na⁺ ions.

Kinetic parameters of dextranacruate were determined in presence of different substrate concentrations (sucrose) at varying pH of 5.2, 6.8, and 8.0. Vmax was calculated as (units/mg Protein) and Km in mM. The enzyme activity was determined after incubation of 30 min at 37 °C as described under material methods. Control = without NaCl

| pH | Assay System | Na⁺ | Vmax (Units/mg Protein) | Km (mM) | R |
|----|-------------|-----|-------------------------|---------|---|
| 5.2| Control     | 0 mM| 1.002                   | 71.92   | 0.90 |
|    | Test        | 2 mM| 0.446                   | 8.228   | 0.98 |
| 6.8| Control     | 0 mM| 2.99                    | 21.358  | 0.93 |
|    | Test        | 2 mM| 1.14                    | 29.914  | 0.80 |
| 8.0| Control     | 0 mM| 0.601                   | 283.22  | 0.98 |
|    | Test        | 2 mM| 0.164                   | 283.14  | 0.78 |

r = Correlation coefficient.

4. Discussion

5–20 mM lithium ions in the basal medium for determination of dextranacruate activity was considered inert as described earlier by Vasseur et al. [11]. The data presented here show that the effects of sodium ion on dextranacruate activity in S. mutans are biphasic. In the acidic pH, the enzyme activity is stimulated by 20–24%, while around neutral and alkaline pH, dextranacruate activity was strongly inhibited by 0.5–2.0 mM sodium. The observed effects of sodium on dextranacruate activity were specific to this ion, since other monovalent cations such as Rb⁺, Cs⁺ and K⁺ were essentially inert. The observed inhibition of dextranacruate was essentially reversible as 86% of the enzyme was recovered upon dialysis. However still the activity was 14% low compared to control which may be due to technical reasons during enzyme dialysis. The observed effect of sodium on dextranacruate are similar to its effects on brush boader sucrase in mammalian intestine. Stimulation of the sucrase activity is also biphasic, sodium ions being a strong activator in the acidic pH (5–6) and an inhibitor in alkaline (7.5–8) pH [12].

Kinetic analysis revealed that sodium ions stimulated dextranacruate activity at pH 5.2, by affecting both the Vmax and Km of the enzyme, indicating a complex mixed type of the kinetics, while at pH 8.0 the enzyme inhibition showed V-type affect, where the Vmax of the enzyme was reduced by 74% without affecting value of Km (283–285) of the enzyme. These findings are in contrast to the effect of sodium on brush boader sucrase, where in the acidic pH, the enzyme activity is stimulated by a marked increase in enzyme Vmax without modifying the enzyme Km [13]. While the enzyme was inhibited in alkaline pH by exhibiting the mixed type complex enzyme kinetics in mice and rabbit intestine [5,6]. These observations indicate considerable differences in the degree of stimulation of dextranacruate in S. mutans and brush boader sucrase in mammalian intestine in response to sodium ions. Although the two enzymes have similar catalytic activity using sucrose as the primary substrate, but the enzyme response to sodium ions are quite distinct which may be related to species difference and their evolutionary origin. The underlying mechanism of the effects of sodium on dextranacruate activity is unknown, but studies with brush boader sucrase have revealed that ionisation of key protons in the active site of enzyme are responsible for the different kinetic mechanism observed as function of pH [14]. Tsumori et al. [15] have reported that Asp, Thr and His residues are essential for the catalytic activity of dextranacruate in S. mutans. It was suggested that Asp acts as nucleophile resulting in the formation of glucosyl-enzyme complex. It is likely that sodium cations may stabilise the intermediate formation in the acidic pH, while it may inhibit the formation of the complex at alkaline pH, due to ionisation of other proton donor groups in the enzyme protein [14].

S. mutans has optimum pH for its growth around 5.2 [16]. Thus the observed stimulation of dextranacruate activity by sodium ions in acidic pH may be of physiological significance for the growth of organism, as S. mutans utilizes the disaccharide solely for its growth and sustenance. The inhibition of dextranacruate by low concentrations of sodium ions (0.5–2.0 mM) at alkaline pH may be useful for blocking the catalytic activity, thus affecting the cariogenic potential of the organism. However such an assertion needs further studies before their full potential can be exploited.

It is also apparent that only the hydrolytic activity of the dextranacruate is influenced by sodium ions, whereas there was no effect on glucosyltransferase activity. It may suggest hydrolytic activity of the enzyme proceeds the transferase activity, as has been suggested by others earlier [17]. Thus inhibiting the hydrolytic activity of dextranacruate by low concentration of Na⁺ ions (0.5–2.0 mM) may be significant in inhibiting the cariogenic activity.

In conclusion, we describe herein that dextranacruate activity in S. mutans, a gram positive bacteria is also affected by sodium ions, but the degree of activation in the acidic pH is markedly small compared to that of mammalian brush boader sucrase. This difference is also reflected in
the kinetic mechanism of the metal ion activation and inhibition of the two enzymes at alkaline pH. Further studies are needed to understand the physiological implication of the new data on metal ion effects on enzyme catalysis in microorganisms.

Compliance with ethical standards
Conflict of interest: All authors declare that there is no conflict of interest with the contents of this article.

Declaration of competing interest
All authors who contributed substantially to the work and participated in the writing of the manuscript, have seen and approved the revised version of this article and no part of the work has been published before. All authors declare that there is no conflict of interest with the contents of this article.

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Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2019.100692.

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