J. Enzyme Inhibition, 1998, Vol. 13, pp. 147–160
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STUDIES ON THE INACTIVATION OF *LEUCONOSTOC MESENTEROIDES* NRRL B-512F DEXTRANSUCRASE BY *o*-PHTHALALDEHYDE: EVIDENCE FOR THE PRESENCE OF AN ESSENTIAL LYSINE RESIDUE AT THE ACTIVE SITE

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(Received 19 July 1995; In final form 23 May 1997)

The kinetics of inactivation of *Leuconostoc mesenteroides* NRRL B-512F dextransucrase by *o*-pthalaldehyde showed that the reaction followed pseudo-first order reaction. The loss of enzyme activity was concomitant with an increase in fluorescence at 417 nm indicating that the inhibition involved the reaction of an ε-amino and a thiol group of the enzyme leading to the formation of an isoindole derivative. The stoichiometry of inactivation showed that one isoindole derivative was formed per enzyme molecule. The substrates sucrose and glucose provided protection against *o*-phthalaldehyde inactivation which was also corroborated by fluorescence studies. Dextransucrase was not inactivated by 5,5′-dithiobis(2-nitrobenzoic acid), showing that the cysteine present in close proximity to the lysine is not essential for enzyme activity. Denaturation of dextransucrase by urea or heat treatment prior to *o*-phthalaldehyde addition resulted in a decrease of fluorescence intensity indicating that the native conformation of the enzyme is essential for isoindole derivative formation. These results established that a lysine residue is present at the active site and is essential for the activity of dextransucrase.

Keywords: Dextransucrase; *Leuconostoc mesenteroides* NRRL B-512F; Chemical modification; *o*-phthalaldehyde

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INTRODUCTION

Leuconostoc mesenteroides NRRL B-512F produces an extra cellular enzyme dextransucrase (Sucrose: 1,6-α-D glucan 6-α-D glucosyl transferase, EC 2.4.1.5) that catalyzes the formation of dextran from sucrose. Many reports have appeared on the purification of dextransucrase[1-5] and extensive investigations have been carried out regarding the structural organization of the active site of the enzyme.[6-8] Separate sucrose and dextran binding domains have been identified in *Streptococcus mutans* dextransucrase.[9] Sucrose-induced conformational changes to align domains into a functional active site have been suggested as the reaction is essentially irreversible.[9] It has been proposed that *Leuconostoc mesenteroides* NRRL B-512F dextransucrase has two sucrose binding sites and one acceptor binding site at the active site.[10] There are few reports on specific amino acid residues present at the active site. Two pertinent amino acid functional groups have been implicated in mechanism of glucosyl transfer from sucrose. An aspartate containing active site peptide bound covalently to glucosyl group was isolated from dextransucrases of *Streptococcus sobrinus*.[11] By chemical modification studies using diethyl pyrocarbonate it was shown that the two essential histidine residues are present at the active site.[12] It has been proposed that the nucleophilic carboxylate group of aspartic acid stabilizes the carbonium ion in equilibrium with the covalent glucosyl–enzyme complex.[11] The imidazolium groups of histidine residues donate protons to the leaving D-fructosyl moieties and the resulting imidazole groups facilitate the formation of the α-(1 $\longrightarrow$ 6)-glucosidic linkage.[12]

Our preliminary studies on the modification of dextransucrase by pyridoxal 5′-phosphate showed that a lysine residue is essential for the enzyme activity.[13] Chemical modification studies have been carried out using the fluorogenic bifunctional reagent, *o*-phthalaldehyde with various enzyme systems for identification of lysine and cysteine as active site residues.[14-21] This reagent specifically binds to the sulfhydryl group of cysteine and ϵ-amino group of lysine and gives a fluorescent, isoindole derivative. Formation of isoindole derivative is possible only when these two functional groups are in close proximity to each other. Thus, it provides vital information about the distance and orientation of these residues at the active site of enzyme. This work reveals the existence of a critical lysine residue at the active site of dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F, based on the chemical modification studies by *o*-phthalaldehyde.

MATERIALS AND METHODS

Material

Leuconostoc mesenteroides NRRL B-512F dextransucrase purified to homogeneity as described previously[5] was used in the present study. *o*-Phthalaldehyde, pyridoxal 5′-phosphate (PLP), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), ethylene diaminetetraacetic-acid (EDTA), L-cysteine and β-mercaptoethanol were obtained from Sigma Chemical Company, USA. All other chemicals used were of highest purity grade commercially available.

Methods

Enzyme Preparation and Activity Assay

Dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F purified by fractionation with polyethylene glycol 400 as reported previously,[5] had a specific activity of 30 U/mg protein. One unit of dextransucrase activity is defined as the amount of enzyme releasing 1 µmole of reducing sugar per min at 30°C and pH 5.2. For all modification reactions dextran-free enzyme was used and the native dextran present in the enzyme was removed by dextranase treatment.[2]

The enzyme activity was determined by measuring the rate of production of reducing sugar. The activity was assayed at 30°C in 0.2 M sodium acetate buffer (pH 5.2). The assay mixture (1.0 ml) containing 10% substrate sucrose in 0.2 M acetate buffer (pH 5.2) and the enzyme solution were incubated at 30°C for 20 min. Aliquots (0.1–0.2 ml), from the reaction mixture were analyzed for reducing sugar by the method of Nelson[22] and Somogyi.[23]

Reaction of o-Phthalaldehyde with Dextransucrase

A solution of *o*-phthalaldehyde was prepared in 0.2 M acetate buffer (pH 5.2) containing 1% distilled methanol. Controls containing the same amount of methanol did not show any effect on enzyme activity. The enzyme (1.2 mg protein/ml) was incubated with the indicated concentrations of *o*-phthalaldehyde at 30°C. At different time intervals 200 µl aliquots were withdrawn from a total volume of 2.0 ml incubation mixture and added to 0.5 ml solution containing 150 µl each of 20 mM cysteine and 5 mM β-mercaptoethanol. This terminated the further reaction of *o*-phthalaldehyde with the enzyme. To the above mixture 0.5 ml of 20% sucrose was added and the mixture incubated for 20 min. The residual enzyme

activity was determined by taking aliquots (0.1–0.2 ml) from the assay mixture (1.0 ml) and analyzing for reducing sugar as described earlier. Control mixtures without *o*-phthalaldehyde were run concurrently.

Fluorescence emission spectra of isoindole derivative resulting from the dextransucrase-*o*-phthalaldehyde reaction were recorded in the presence and absence of β-mercaptoethanol by excitation at wavelength 337 nm. Fluorescence intensity enhancement due to isoindole derivative formation was measured at different time intervals without terminating the enzyme–inhibitor reactions with cysteine and β-mercaptoethanol.

The stoichiometry of the reaction was determined by incubating the enzyme (1.2 mg/ml) in 0.2 M acetate buffer (pH 5.2) with 5 mM *o*-phthalaldehyde for 30 min at 30°C. The concentration of isoindole derivative and enzyme were calculated using the absorbance coefficient of 7660 $M^{-1} cm^{-1}$ at 337 nm[19] and Lowry's method,[24] respectively. The molecular weight of dextransucrase was taken[5] as 188,000.

Fluorescence emission spectra were scanned on a luminescence spectrometer, Perkin Elmer (Model LS 50B). Absorbance was measured on a UV–vis spectrophotometer, Shimadzu (Model UV-160A). Both fluorescence and the absorbance were recorded in cuvettes of 1 cm light path at 30°C.

Effect of Sucrose and Acceptor Substrates on Inactivation of Dextransucrase by o-Phthalaldehyde

For protection experiments, the enzyme (1.2 mg protein/ml, 30 U/mg) was incubated with 50 mM EDTA in 0.2 M acetate buffer (pH 5.2) at 30°C for 30 min. The sucrose was then added and the mixture incubated for another 15 min at 30°C prior to the addition of 5 mM *o*-phthalaldehyde. After 30 min the residual activity was determined following the same procedure as described earlier. The assay mixture contained 50 mM of Ca^{2+} for reactivation of the enzyme. The enzyme preincubated with EDTA followed by treatment with Ca^{2+} without *o*-phthalaldehyde was used as control.

The enzyme (1.2 mg protein/ml) was incubated with 50 mM and 100 mM glucose for 15 min at 30°C prior to the addition of 5 mM *o*-phthalaldehyde. After 30 min the residual activity was determined. The appropriate controls without *o*-phthalaldehyde and with glucose were run concurrently.

Effect of Prior Incubation of Dextransucrase with DTNB and PLP on the Modification of Enzyme by o-Phthalaldehyde

The enzyme (1.2 mg protein/ml) was incubated with 5 mM 5,5′-dithiobis (2-nitrobenzoic acid; DTNB) for 15 min prior to incubation with 5 mM

o-phthalaldehyde at 30°C. The incubation of enzyme with two inhibitors was characterized by monitoring the fluorescence emission spectra on excitation at 337 nm before and after the incubation with *o*-phthalaldehyde. Similarly, the enzyme (1.2 mg protein/ml) was first incubated with 30 mM pyridoxal 5′-phosphate (PLP) at 30°C for 1 h followed by incubation with 5 mM *o*-phthalaldehyde for 30 min. The fluorescence emission spectra were recorded with excitation at 337 nm, before and after the dialysis of the above incubation mixture.

Effect of Dextransucrase Denaturation on o-Phthalaldehyde Reaction

The enzyme was denatured by incubation with 8 M urea or kept in boiling water for 2 min and incubated with 5 mM *o*-phthalaldehyde and fluorescence spectra were recorded after 30 min of incubation.

RESULTS

Kinetics of *o*-Phthalaldehyde Inactivation of Dextransucrase

Dextransucrase was rapidly and irreversibly inactivated by *o*-phthalaldehyde (Figure 1). The residual enzyme activity was plotted on a semi-logarithmic scale against time of incubation. The linear relationships obtained during the initial phase of activity loss indicated that the inactivation followed pseudo-first order kinetics (Figure 1). The second order rate constant of $8.75\,M^{-1}\,min^{-1}$ was obtained by plotting the observed pseudo-first order rate constants against the concentrations of *o*-phthalaldehyde (Figure 1, Inset). The modification of dextransucrase with *o*-phthalaldehyde led to an enhancement in fluorescence intensity at 417 nm, which was proportional to the degree of inactivation (Figure 2).

Spectral Analysis of Modified Dextransucrase by *o*-Phthalaldehyde in the Presence and Absence of β-mercaptoethanol

A fluorescence emission spectra of *o*-phthalaldehyde-modified dextransucrase showed a maximum at 417 nm (λ_{em}) upon excitation at 337 nm (Figure 3). This was consistent with the formation of an isoindole derivative which involves the participation of proximal thiol and ε-amino groups of cysteine and lysine, respectively.[15,16] The molar transition energy (E_T) was calculated by the following equation:[15]

$$E_T = 2.985\lambda_{em} = 1087.28.$$

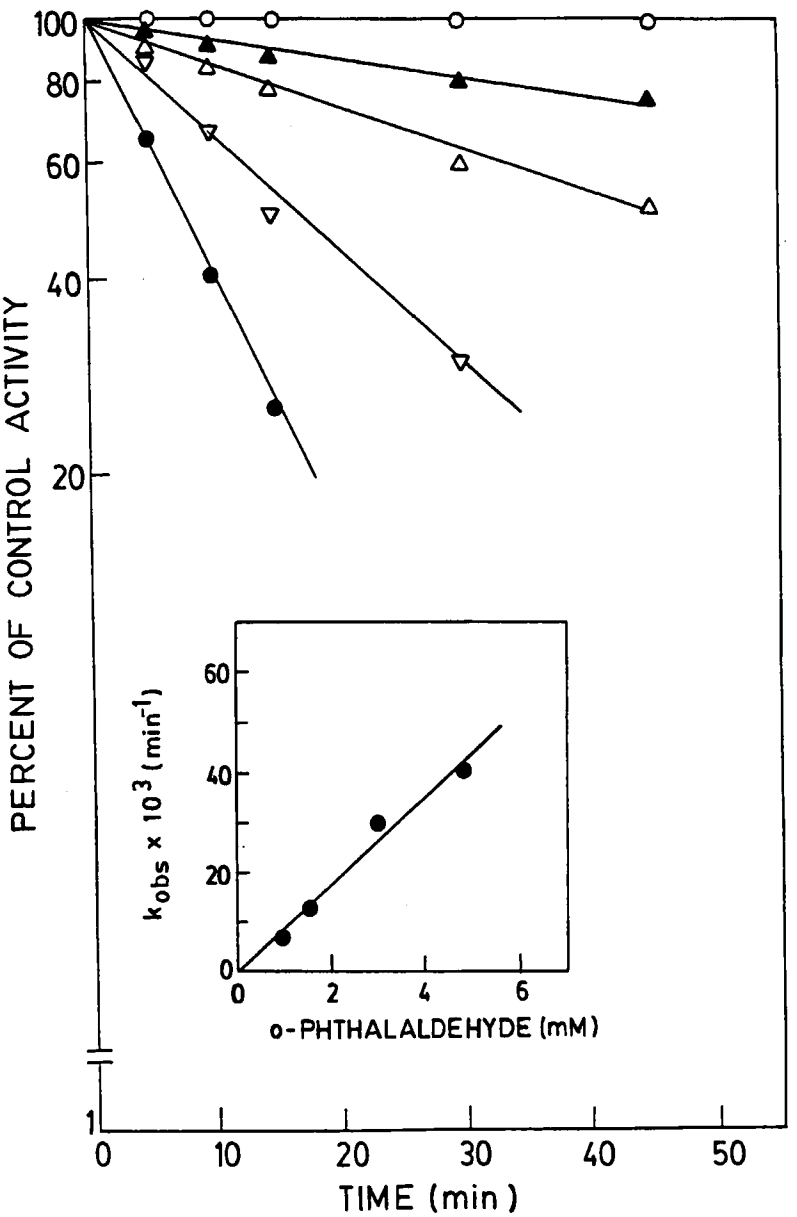

FIGURE 1 *Kinetics of dextransucrase inactivation by o-phthalaldehyde.* The enzyme (1.2 mg/ml, 30 U/mg protein) in 0.2 M acetate buffer (pH 5.2), was incubated with 0 (○), 1 (▲), 1.5 (△), 3 (▽) and 5 mM (●) *o*-phthalaldehyde at 30°C. Aliquots were withdrawn at the indicated time intervals and the residual activity was determined as described in "Materials and Methods". Inset: a plot of the observed pseudo-first order rate constant vs initial *o*-phthalaldehyde concentrations, from which a second order rate constant was obtained.

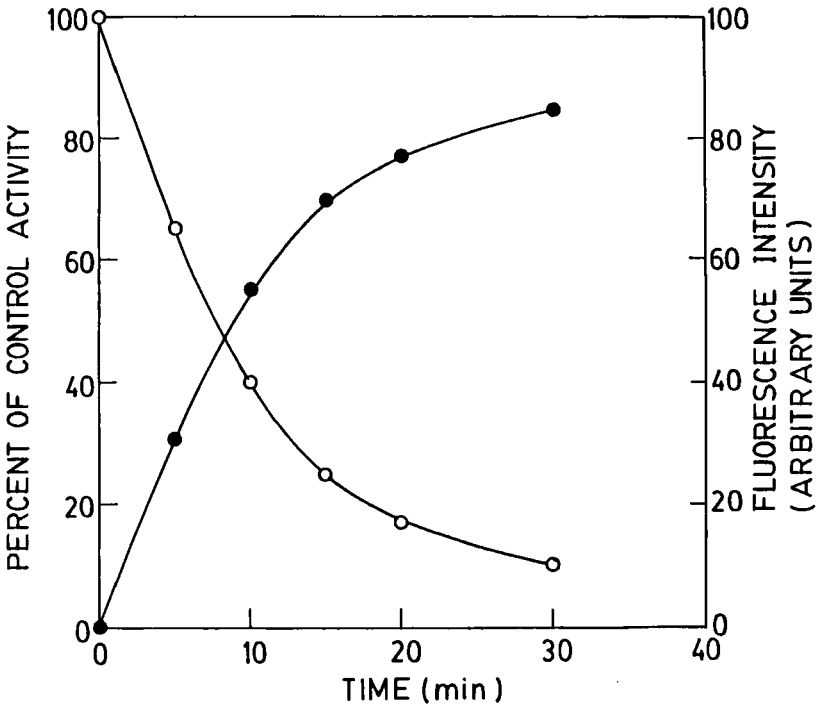

FIGURE 2 *Time course of inactivation of dextransucrase* (○) *and increase in fluorescence intensity* (●) *on excitation at* 337 *nm on treatment with o-phthalaldehyde.* Dextransucrase (1.2 mg/ml) was incubated with 5 mM *o*-phthalaldehyde in 0.2 M acetate buffer (pH 5.2). In another set of experiments enzyme activity was measured as described in "Materials and Methods".

The calculated molar transition energy of dextransucrase and *o*-phthalaldehyde adduct was found to be 157.5 kJ/mol which was close to that of synthetic isoidole in dioxane[15,16] indicating thereby that the microenvironment around the cysteine and lysine residues involved in isoindole formation is relatively hydrophobic in nature. The fluorescence intensity was much higher in the presence of β-mercaptoethanol than in its absence showing that many more lysine residues are modified. However, the kinetics of inactivation by *o*-phthalaldehyde in the presence of β-mercaptoethanol showed no significant differences in the rate of inactivation. These observations led to the conclusion that lysine residues which are modified only in the presence of β-mercaptoethanol do not contribute to the loss of activity and thus are not critical for enzyme activity. Further, the fluorescence emission spectra of the enzyme modified with *o*-phthalaldehyde in the presence of β-mercaptoethanol showed a maxima at 446 nm (λ_{em})

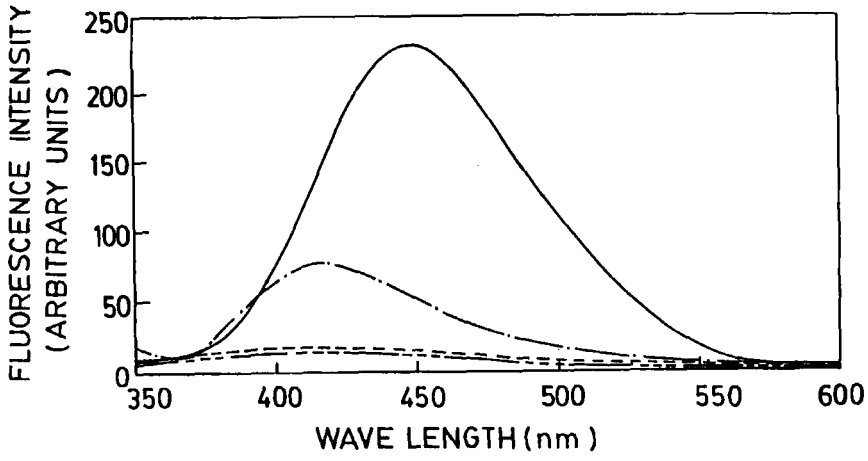

FIGURE 3 *Fluorescence emission spectra of dextransucrase-o-phthalaldehyde adduct in the presence and absence of β-mercaptoethanol.* The enzyme (1.2 mg/ml) in 0.2 M acetate buffer (pH 5.2) was incubated with 5 mM *o*-phthalaldehyde for 30 min at 30°C. In another set of experiments the enzyme preincubated with 5 mM β-mercaptoethanol was treated with 5 mM *o*-phthalaldehyde. The resulting isoindole derivatives were characterized by fluorescence emission spectrum in the presence (—) and absence (– - –) of β-mercaptoethanol with excitation wavelength at 337 nm. The emission spectra of pure enzyme (--- - - ---) and in the presence of β-mercaptoethanol (- - - -) were recorded with excitation at 337 nm. For details see "Materials and Methods".

upon excitation at 337 nm (Figure 3). The calculated value of E_T for this system was 244 kJ/mol which is closer to the value of isoindole in methanol,[15,16] thus, showing that the resulting isoindole derivatives are in a hydrophilic environment.

Stoichiometry of Dextransucrase Inactivation by *o*-Phthalaldehyde

The stoichiometry of inhibition by *o*-phthalaldehyde was determined from the absorbance increase at 337 nm. It was found that 80% of inactivation occurred within the first 15 min of incubation. Approximately, one mol of isoindole derivative was formed per mol of enzyme (Table I).

Effect of Sucrose and Acceptor Substracts on Dextransucrase Inactivation by *o*-Phthalaldehyde

The conventional protection experiments with only sucrose were not possible, as dextransucrase undergoes single substrate reaction. However, the property of enzyme inhibition by EDTA and reversal by Ca^{2+} ions was utilized for determining the effect of sucrose on *o*-phthalaldehyde

TABLE I *Quantitative analysis of dextransucrase inactivation by o-phthalaldehyde.* Different concentrations of dextransucrase were incubated with 5 mM *o*-phthalaldehyde for 15 min, which resulted in 80% of inactivation of the enzyme. The number of isoindole derivatives formed per enzyme molecule was calculated as described in "Materials and Methods"

Concentration of dextransucrase (μM)	*Absorbance at* 337 nm	*Number of isoindole derivatives formed/enzyme molecule*
1.0	0.007	0.91
2.7	0.020	0.97
4.0	0.030	0.98
4.8	0.038	1.03

TABLE II *Effect of sucrose and glucose on dextransucrase modification by o-phthalaldehyde.* The reagents shown were incubated with the enzyme (1.2 mg/ml) for the indicated time period followed by incubation with 5 mM *o*-phthalaldehyde for 30 min. Appropriate controls in each case without *o*-phthalaldehyde were run concurrently. For details see "Materials and Methods"

Reagent	*Activity* (%)
None	10
EDTA (50 mM, 30 min)	45
Sucrose (100 mM, 15 min)	90
Sucrose (200 mM, 15 min)	93
Sucrose (300 mM, 15 min)	97
Glucose (50 mM, 15 min)	85
Glucose (100 mM, 15 min)	95

inactivation.[1] The enzyme on incubation with 50 mM EDTA at 30°C resulted in 55% loss of activity in 30 min (Table II). The addition of 50 nM Ca^{2+} to the EDTA-inactivated dextransucrase resulted in almost complete reactivation of the enzyme. This approach was used to carry out the protection experiments, in presence of sucrose by quenching the activity of the enzyme and then reactivating it. The results of the effect of sucrose on dextransucrase inactivation by *o*-phthalaldehyde are shown in Table II. A concentration of 300 nM of sucrose provided the maximum protection to the enzyme against inactivation by *o*-phthalaldehyde. Protection of enzyme by sucrose from inactivation was further corroborated by the decrease in the fluorescence intensity (Figure 4).

D-glucose has been reported to be an acceptor substrate and is also used as the active site protecting reagent for dextransucrase.[26] D-glucose is nonreactive and meets the structural requirements for binding to the active site of the enzyme. Approximately, 95% of the enzyme activity was retained in the presence of 100 mM glucose against *o*-phthalaldehyde inactivation (Table II). These results indicated that the inactivation of dextransucrase

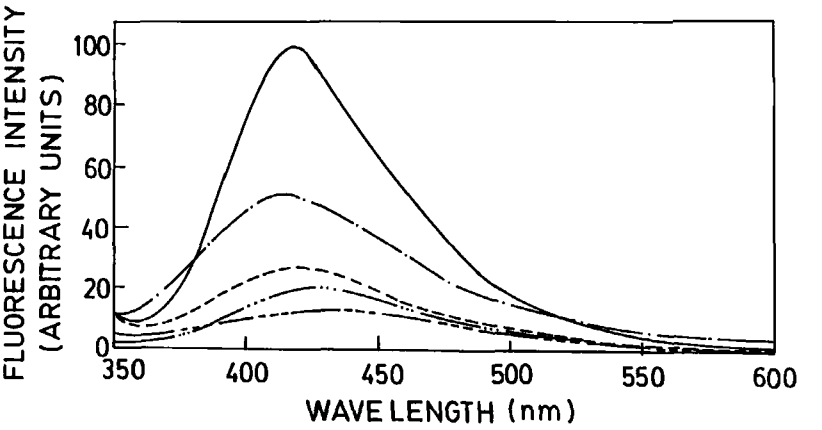

FIGURE 4 *Effect of substrate sucrose and denaturants on the binding of o-phthalaldehyde with dextransucrase.* The enzyme (1.2 mg/ml) was preincubated with 50 mM EDTA for 30 min, followed by incubation with 300 mM substrate sucrose for 15 min and finally with 5 mM *o*-phthalaldehyde in 0.2 M acetate buffer (pH 5.2) for 30 min at 30°C. A control was run in parallel without the sucrose. The fluorescence spectra of the control (—) and with 300 mM sucrose (— - —) were recorded with excitation wavelength 337 nm. The emission spectra of pure enzyme (– - - - –) and the enzyme denatured by urea (- - - - -) or heat (– - - - –) followed by *o*-phthalaldehyde treatment are also shown. For details see "Materials and Methods".

by *o*-phthalaldehyde has resulted from the modification of lysine and cysteine residues located at the active site of the enzyme.

Characterization of the Cysteine and Lysine Residues Involved in the *o*-Phthalaldehyde Reaction

The enzyme showed no inactivation on treatment with thiol specific inhibitors. The enzyme on incubation with DTNB for 15 min followed by the incubation with *o*-phthalaldehyde for 30 min lost 85% of its activity (Table III). This clearly showed that even in the presence of DTNB, *o*-phthalaldehyde was able to bind to the lysine leading to the inactivation of the enzyme. It was interesting to note that dextransucrase preincubated with DTNB followed by treatment with *o*-phthalaldehyde did not show any fluorescence on excitation at 337 nm. This was due to the binding of DTNB to cysteine thereby preventing the formation of an isoindole derivative. These results indicated that the cysteine is present in close proximity to the lysine and is involved in the isoindole derivative formation but is not critical for the activity of the enzyme.

TABLE III *Effect of DTNB on the inactivation of dextransucrase by o-phthalaldehyde.* The enzyme (1.2 mg protein/mg) was preincubated with DTNB in 0.2 M acetate buffer (pH 5.2) at 30°C. This was followed by treatment with *o*-phthalaldehyde for the indicated time interval. Aliquots (0.2 ml) from incubation mixtures were analysed for enzyme activity as described in "Materials and Methods"

Reagent	*Activity* (%)
None	100
o-phthalaldehyde (5 mM, 30 min)	10
DTNB (5 mM, 30 min)	95
DTNB (5 mM, 15 min) + *o*-phthalaldehyde (5 mM, 30 min)	15

It has been reported that *o*-phthalaldehyde can react with a primary amino group in the absence of thiol compounds leading to the formation of non-fluorescent products.[27] The results suggested that *o*-phthalaldehyde reacted with the specific lysine residue even when the cysteine was blocked by DTNB to give a non-fluorescent adduct, which inactivated the enzyme. However, in the absence of DTNB, inactivation of dextransucrose by *o*-phthalaldehyde showed a good correlation with isoindole formation (Figure 2). These results clearly indicated that the cysteine is present in close proximity to the lysine and is not essential for the activity of the enzyme.

Experiments with prior incubation of dextransucrase with PLP followed by *o*-phthalaldehyde incubation, were performed in order to ascertain whether these two inhibitors are binding to the same lysine residue that leads to enzyme inactivation. It is generally known that the inhibition by PLP can be reversed by dilution or dialysis. The enzyme on incubation with PLP followed by *o*-phthalaldehyde without terminating the reaction by cysteine and β-mercaptoethanol, did not show any fluorescence, but after dialysis it showed a fluorescence emission maximum at 417 nm with much reduced intensity as compared to the control (Figure 5). Similar results were obtained even when lower concentrations of PLP were used. This observation indicated that both the inhibitors are binding to the same lysine residue that is involved in the catalytic activity of dextransucrase. All these results lead to the conclusion that there is one lysine residue which is essential for enzyme activity.

Effect of Enzyme Denaturation on the Modification by *o*-Phthalaldehyde

Dextransucrase when treated with urea or heat followed by incubation with *o*-phthalaldehyde was observed to give a marked decrease in the fluorescence emission intensity as compared to the native enzyme *o*-phthalaldehyde adduct (Figure 4). These results clearly indicate that the proximal

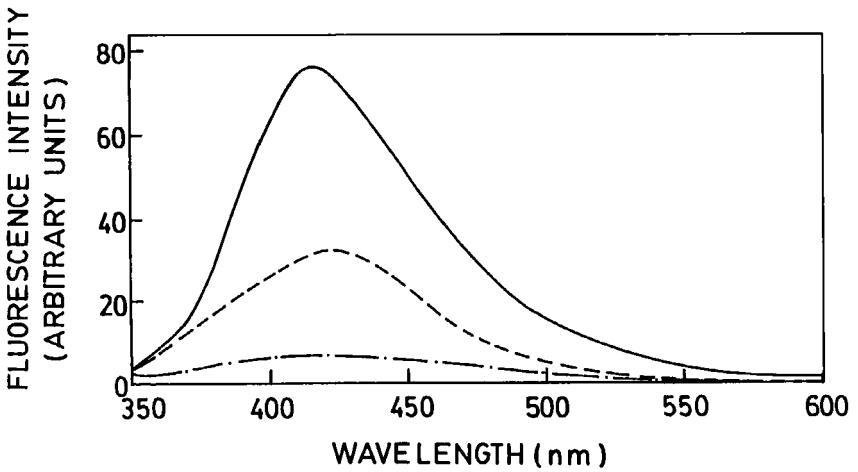

FIGURE 5 *Effect of PLP pretreatment of dextransucrase on fluorescence emission spectra of dextransucrase-o-phthalaldehyde adduct.* The enzyme (1.2 mg/ml) in 0.2 M acetate buffer (pH 5.2) was incubated with 30 mM PLP for 1 h followed by treatment with 5 mM *o*-phthalaldehyde for 30 min at 30°C. The fluorescence emission spectra were recorded with excitation wave length, before (— - —) and after (- - - - -) dialysis of the incubation mixture. A control (—) with the enzyme treated with 5 mM *o*-phthalaldehyde for 30 min was run in parallel without PLP pretreatment.

integrity of the lysine and cysteine residues at the active site of the native enzyme is essential for *o*-phthalaldehyde reaction.

DISCUSSION

Chemical modification reagents can be used successfully to identify amino acid residues that are important for enzyme activity provided that the following two criteria are fulfilled: (1) chemical modification of these amino acid residues must result in a loss of activity, (2) this inactivation must be prevented by substrates or substrate analogues. Dextransucrase was rapidly inactivated by *o*-phthalaldehyde and the inactivation followed pseudo-first order kinetics. Spectrofluorometric results with dextransucrase and *o*-phthalaldehyde indicated the presence of a relatively hydrophobic environment at the active site. The substrate sucrose and acceptor substrate glucose protected the enzyme against *o*-phthalaldehyde inactivation indicating that the lysine and cysteine residues are present at the active site. The stoichiometry of the reaction of dextransucrase with *o*-phthalaldehyde showed that one isoindole derivative is formed per enzyme molecule. The calculated molar transition energy 157.5 kJ/mol was found to be close

to the value 154 kJ/mol obtained for synthetic isoindole in the non-polar hydrophobic environment of dioxane.[15,16] The hydrophobicity at the active site of dextransucrase dictates that the ε-amino group of lysine remains in a deprotonated form to permit reaction with *o*-phthalaldehyde.[18] The pK_a determination for the reaction of dextransucrase-*o*-phthalaldehyde probably could have provided more information. But, the pH-dependence of the inactivation process could not be determined as the enzyme itself, to a large extent, lost activity even at a slightly higher or lower pH than 5.2. However, the formation and characterization of the isoindole derivative at pH 5.2 suggested that the ε-amino group of lysine reacting with *o*-phthalaldehyde is in the deprotonated form, corroborating the hydrophobic nature of the microenvironment of lysine at the active site. The kinetic data in conjunction with fluorescence suggested that the lysine residues which are modified only in the presence of β-mercaptoethanol are not critical for activity of the enzyme. Thus only one specific lysine residue which is proximal to cysteine is essential for the activity of dextransucrase.

Dextransucrase was not inactivated by thiol specific inhibitors indicating that the cysteine involved in the *o*-phthalaldehyde reaction leading to isoindole formation is not required for activity. Further, the dextransucrase on preincubation with DTNB followed by treatment with *o*-phthalaldehyde did not exhibit any fluorescence on excitation at 337 nm. However, the inactivation of the enzyme was still observed as *o*-phthalaldehyde was able to react with the specific lysine residue and form a non-fluorescent product, when the proximal cysteine was blocked by DTNB. This clearly indicated that the cysteine in close proximity to the lysine is not essential for enzyme activity. Dextransucrase pretreated with PLP followed by incubation with *o*-phthalaldehyde showed that both these inhibitors are binding to the same specific lysine residue that is essential for the enzyme activity.

The formation of the isoindole derivative need not depend on the closeness of lysine and cysteine residues in the primary structure, but, rather on the specific proximity of these residues in the tertiary structure of the enzyme.[15] The fluorescence intensity of the isoidole derivative, formed due to the reaction of *o*-phthalaldehyde with denatured dextransucrase was lowered as compared to the native form of enzyme. Thus, it can be concluded that alteration in the native conformation of the enzyme disrupts the favorable orientation of lysine and cysteine residues at the catalytic centre for the *o*-phthalaldehyde reaction.

The results of the present chemical modification studies using *o*-phthalaldehyde conclusively demonstrated the presence of a lysine residue at the

active site that is essential for the activity of dextransucrase, whereas, the proximal cysteine also present at the active site is not essential for enzyme activity. The results showed that the essential lysine residue is in some way involved in maintaining the activity of dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F. However, it is not clear whether this residue is directly associated with enzyme catalysis, with binding of the substrate, or with maintaining the enzyme in an active conformational state.

Acknowledgement

We are thankful to Dr. Leela Iyengar for helpful discussions.

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