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Note

The action pattern and subsite map of *Streptococcus mutans* Kl-R dextranase*

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Streptococcus mutans Kl-R dextranase is thought to modify, and possibly to regulate the nature of the dextrans synthesized by the extracellular D-glucosyltransferase of *S. mutans*[1]. Because of its possible involvement in dental caries, we have explored the action pattern of this enzyme on a series of end-labeled oligosaccharides. These data were used to generate a subsite map[2,3] of the enzyme that could predict the action pattern of the enzyme with remarkable precision.

The enzyme was purified, as described earlier, from cell-free filtrates of *S. mutans* Kl-R that had grown in batch culture with the pH maintained at 6.0. Two homologous series of isomaltose oligosaccharides, labeled with D-[U-^{14}C]glucose at opposite ends, were used as substrates. Isomaltose saccharides labeled at the non reducing terminus were prepared by coupling [U-^{14}C]sucrose and unlabeled isomaltose saccharide acceptor by means of a D-glucosyltransferase[4] of *S. mutans* OMZ176. The specific activity of these substrates was 8-fold higher than those described previously. The substrates labeled at the reducing end[5] were prepared by transferring isomaltosyl residues from dextran to D-[U-^{14}C]glucose[6] with *Arthrobacter globiformis* T6 exo-dextranase.

The digests used to determine action patterns contained labeled substrate (0.2mM), sodium citrate buffer (5mM), and an amount of enzyme that depended on the size of the substrate, being 0.18 i.u. per ml for isomaltotetraose (IM_4) and IM_5, and 0.09 i.u. per ml for IM_6–IM_9. Incubation times (at 35° at pH 5.5) for IM_6–IM_9 were in the range 10–60 min, whereas IM_4 and IM_5 were digested for periods up to 24 and 3 h, respectively. At intervals, aliquots were boiled, and applied to Whatman No. 3 MM chromatography paper, and the products and unreacted substrate were separated by irrigation with nitromethane–ethanol–water (41:36:23) as described elsewhere[7]. Radioautograms were then prepared by using X-ray film, and after excision of the spots, the radioactivity of the sugars was measured by liquid-scintillation spectrometry[7]. Bond-cleavage frequencies were obtained by plotting the ratio: radio-

*Dedicated to Professor Dexter French, friend and mentor, on the occasion of his 60th birthday.

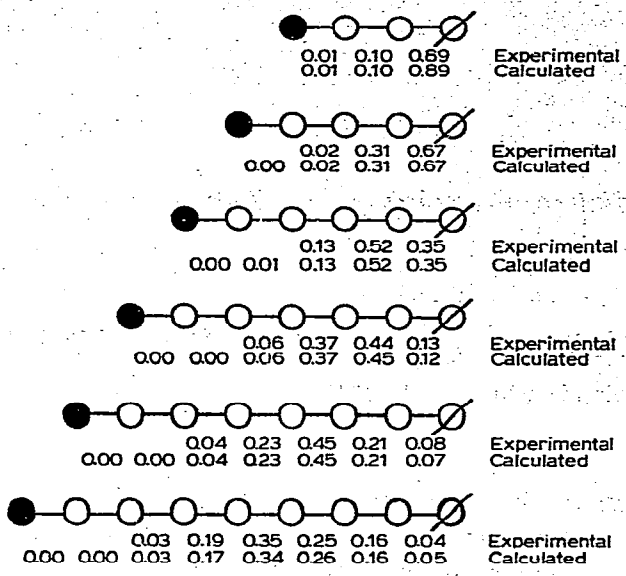

Fig. 1. Experimental and calculated bond-cleavage frequencies for *S. mutans* dextranase. ○, D-Glucose residue; ∅, "reducing" D-glucose residue; ●, D-[^{14}C]glucose residue; ——, (1 → 6)-α-D-glucosidic linkage.

activity due to each sugar (*i*-mer) in a sample/total radioactivity in the sample, against the extent of reaction[8]. The slope of each line represented[8] the bond-cleavage frequency for the bond resulting in the product of chain-length *i*.

The experimental bond-cleavage frequencies for IM_4–IM_9 labeled at the non-reducing end are shown in Fig. 1. Similar values were obtained for IM_5[5], IM_6, and IM_7 (not shown) labeled at the reducing end, indicating that multiple attack, secondary attack on the products, and bimolecular reactions did not significantly influence the product ratios.

The experimental bond-cleavage frequencies (bcf) may be utilized by a computer algorithm to generate a subsite map[9,10]. The process of subsite mapping is basically a problem of optimization. A brief description of the process follows. For a given depolymerase model, the number of subsites, the position of the catalytic amino acids, and the experimental bcf are incorporated into the computer algorithm. The computer then finds the subsite binding-affinities that minimizes a sum of squares of errors, Q. For this investigation we defined Q by Eq. *1*

$$Q_{bcf} = \sum_i W_i^2 (bcf_{i,expt.} - bcf_{i,calc.})^2 \quad , \qquad (1)$$

where w_i is the weighting factor set[9] at $\frac{1}{0.03}$.

The minimum values of Q, $Q_{min,bcf}$, for a variety of possible depolymerase models are given in Table I. The depolymerase model selected as the one most likely to represent the actual enzyme binding-site gives the smallest significant $Q_{min,bcf}$. If

TABLE I

COMPUTER SUBSITES MODELS OF *S. mutans* DEXTRANASE

Model number[c]	*I*	*II*	*III*	*IV*	*V*	*Catalytic*[b] *site* ↓ *VI*	*VII*	*VIII*	*IX*	*X*	*XI*	$Q_{min,bcf}$[a]
11/7	×	×	×	×	×			×	×	×	×	1.7
10/7	×	×	×	×	×			×	×	×		2.1
9/7	×	×	×	×	×			×	×			2.2
8/7	×	×	×	×	×			×				25
7/7	×	×	×	×	×							132
					Catalytic[b] *site* ↓							
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VII*	*IX*	*X*	*XI*	
11/6	×	×	×	×			×	×	×	×	×	2.3
10/6	×	×	×	×			×	×	×	×		2.3
9/6	×	×	×	×			×	×	×			4.1
8/6	×	×	×	×			×	×				9.1
9/5		×	×	×			×	×	×	×		81

[a]The free energies of the subsites marked × are optimized by the computer algorithm to minimize Q_{bcf}. [b]Bond-cleavage frequencies do not furnish information about the two sites adjacent to the catalytic site. [c]Model number indicates the number of subsites optimized (top number) and the number of the subsite to the right of the catalytic site (bottom number).

two depolymerase models do not have significantly different $Q_{min,bcf}$ values, as judged by the *F* test, the one having the smaller number of subsites is favored[9,10]. By these criteria, the 9/7 model, having 9 subsites and the catalytic site to the left of subsite 7, gives the best fit to the experimental data.

The optimized binding-energies for sites I–V and VIII and IX for the 9/7 map, as calculated by the computer, are shown in Fig. 2. These binding affinities are only apparent, as they may contain a contribution from microscopic rate-coefficients[9,10]. The small, positive, interaction energy at subsite I is statistically significant, but it is too weak to be described as a barrier site. It probably reflects some minor, unfavorable contacts between enzyme and substrate just beyond the edge of the binding region.

When the subsite histogram in Fig. 2 was used to compute the bond-cleavage frequencies for the substrates of chain length 4 to 9, the agreement with the experimental values was excellent (see Fig. 1). The close agreement between each computed and experimental value suggests that microscopic hydrolytic coefficients are not strongly influenced by positional isomerization[9,10].

The large size of the binding region of *S. mutans* dextranase (9 subsites) seems to be typical of the values reported for other endo-amylases and dextranases. Richards and Streamer[11] proposed that *Pseudomonas sp.* UQM733 dextranase D_1 has six subsites. Janson[12] predicted that the dextranases of *Cytophaga johnsonii* and *Arthrobacter* sp. contain eight and ten (or more) subsites, respectively.

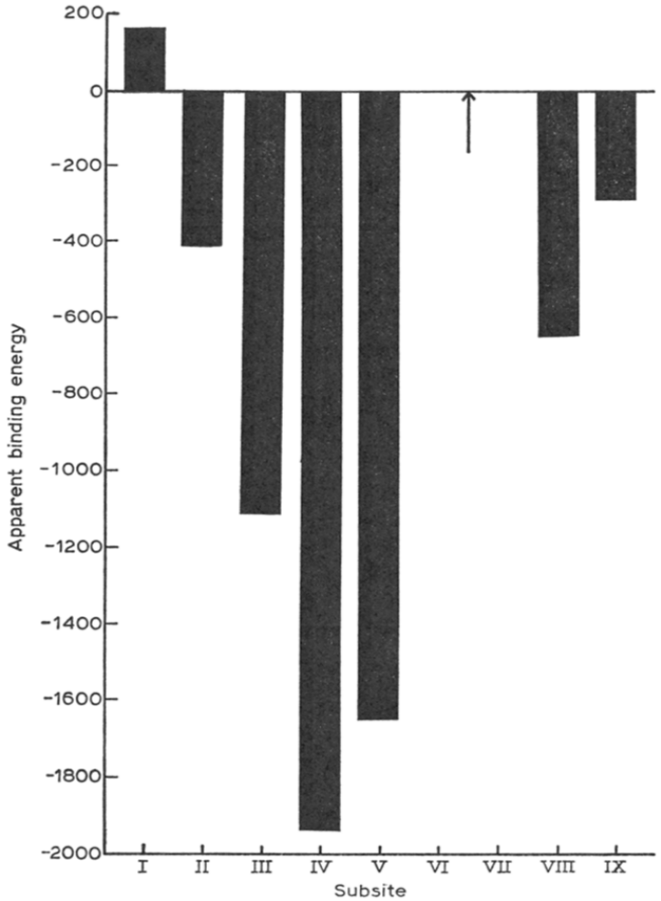

Fig. 2. Apparent subsite-binding energies (cal/mol) for *S. mutans* dextranase. The arrow represents the position of the catalytic amino acids. Product ratios cannot furnish any information about the subsites adjacent to the catalytic amino acids.

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