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SOME DILUTE-SOLUTION PARAMETERS OF THE LEVAN OF *Streptococcus salivarius* IN VARIOUS SOLVENTS*

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ABSTRACT

The intrinsic viscosities, weight-average molecular weights ($\bar{M}_w$), and radii of gyration $[(\overline{R_g^2})_z^{\frac{1}{2}}]$ of *Streptococcus salivarius* levan in various solvents were respectively obtained from viscosity and light-scattering measurements. The data showed that the levan in water is not aggregated by hydrogen bonds, and that the values of both the refractive index and $(\overline{R_g^2})_z^{\frac{1}{2}}$ are lower in water than in aqueous solutions of urea. Urea may break intramolecular hydrogen-bonds, *e.g.*, between branches, allowing the molecule to expand.

INTRODUCTION

Levan, a polysaccharide almost entirely composed of D-fructofuranosyl residues joined by β-D-(2→6) bonds[2], is found in plants[3], and in the extracellular substances of some bacteria[4]; is a type generally branched through β-D-(2→1) bonds. The levan produced from dental plaque[5] is of considerable interest, as it has been implicated in dental caries and periodontal diseases.

The elution fractionation, chemical analysis, and intrinsic viscosities of *Streptococcus salivarius* levan in water and in dimethyl sulfoxide have been reported[6]. Various solution parameters obtained from light-scattering and sedimentation analysis showed that the molecular weights of the fractions varied[7,8] from ~18 × 10⁶ to 60 × 10⁶. The *S. salivarius* levan used in these studies was found to have a highly branched, compact structure in water. It was shown[9] that the molecular weight of levan produced by *S. salivarius* is related to the pH of the medium. Further kinetic studies on the hydrolysis of levan demonstrated[10] that the degradation is non-random.

The intent of this paper is to examine the effect of various solvents on various hydrodynamic parameters, in order to shed additional light on the behavior, *e.g.*, aggregation, of levan in solution.

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*Levans. Part VII. For Part VI, see ref. 1.

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EXPERIMENTAL

Materials — The native-levan sample L3 was the same as that employed formerly[10], having a weight-average molecular weight of $23\ 7 \times 10^6$

Methods — Procedures for measuring intrinsic viscosities and light-scattering were reported[7 8] earlier

The refractive index (n) of water at 546 1 nm at 25° was obtained from table II of the article by Johnson and Smith[11], and the value of n at 57° was calculated by using the value[11] of $-1\ 1 \times 10^{-4}$ deg^{-1} for dn/dt Refractive indices at 589 3 nm for the solvent systems employed were obtained from a Handbook[12], these were converted into values at 546 1 nm by graphical interpolation, using the simplified Cauchy formula for solvents of known refractive indices at these wavelengths[11 12] The correction to n was small, 0 01–0 02, and the values obtained for the solvents and solvent-systems are listed in Table I

TABLE I

INCREMENT IN REFRACTIVE INDEX, dn/dc, OF *Streptococcus salivarius* LEVAN L-3 IN VARIOUS SOLVENTS AT 546 1 nm AND 25°

Solvent	*Refractive index, n, of solvent*	*dn/dc*
H_2O	1 334	0 142
H_2O at 57°	1 330	0 144
0 1M NaCl	1 335	0 141
M NaCl	1 344	0 135
4M NaCl	1 371	0 118
4M Urea	1 369	0 120
6M Urea	1 385	0 110
8M Urea	1 402	0 099
11 9 Ethanol–water	1 362	0 124
6M Urea in 11 9 ethanol–water	1 386	0 109

The specific increment in refractive index (dn/dc) for levan in different solvent-systems was calculated from the modified Gladstone–Dale equation[13], namely,

$$(\mathrm{d}n/\mathrm{d}c)_2 = (\mathrm{d}n/\mathrm{d}c)_1 - \bar{v}(n_2 - n_1)\,, \qquad (1)$$

where subscripts 1 and 2 refer to solvents, and $\bar{v}$ is the partial, specific volume of the solute For levan in water, dn/dc and $\bar{v}$ are 142 μL g^{-1} and 0 63 mL g^{-1}, respectively[6 7] The values of dn/dc calculated for levan in the other solvents, under the assumption that $\bar{v}$ is constant, are listed[14] in Table I

For 6M urea in 11 9 ethanol–water, the refractive index was obtained from the empirical Gladstone–Dale equation[11], namely,

$$(n-1)/\rho = w_1(n_1-1)/\rho_1 + w_2(n_2-1)/\rho_2\,, \qquad (2)$$

where w_1 and w_2, ρ_1 and ρ_2, and n_1 and n_2 refer to the weight fractions, densities and refractive indices of 11 9 ethanol–water and urea, respectively, and n is the refractive index of the water–ethanol–urea mixture ρ is the density of the water–ethanol–urea mixture, 0 995 g mL^{-1}, which was estimated by taking the average of the values[12] for 6M urea in water and of 11 9 ethanol–water

RESULTS AND DISCUSSION

Figs 1 and 2 show typical viscosity and light-scattering plots, from which $[\eta]$ and both $\bar{M}_w$ and $\overline{(R_g^2)}_z^{\frac{1}{2}}$ are respectively obtained The weight-average molecular

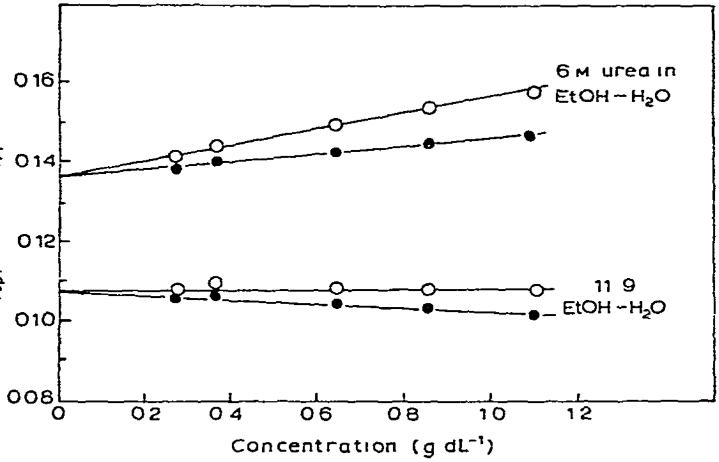

Fig 1 Intrinsic-viscosity plots of levan L-3 in 11 9 ethanol–water and 6M urea in 11 9 ethanol–water [Open circles η_{sp}/c, filled circles, ln (η_r/c)]

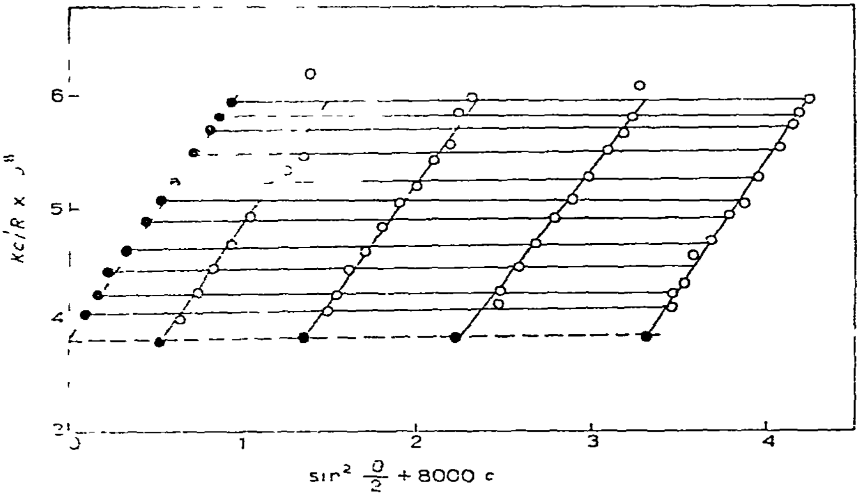

Fig. 2 Zimm plot of levan L-3 in 4M aqueous urea (concentration in g mL^{-1})

weight ($\bar{M}_w$), the intrinsic viscosity [η], and the z-average, root-mean-square radii of gyration, $(\overline{R_g^2})_z^{\frac{1}{2}}$, of levan in various solvent-systems are shown in Table II. The $\bar{M}_w$ is, within experimental error, $\sim 24 \times 10^6$, the value reported[10] for this levan sample The somewhat lower and higher values in 4M sodium chloride and 8M urea, respectively, may be attributed to approximations made in calculating the specific increment in refractive index from eq *1* and, perhaps, to adsorption of ions

TABLE II

VISCOSITY AND LIGHT-SCATTERING PARAMETERS OF *Streptococcus salivarius* LEVAN L-3 IN VARIOUS SOLVENTS AT 25°

Solvent	$\bar{M}_w \times 10^{-6}$	[η] (*dL* g^{-1})	$(\overline{R_g^2})_z^{\frac{1}{2}}$ (*nm*)
H_2O	23 7	0 134	34 9
H_2O at 57°	24 3	0 188	41 6
0 1M NaCl	26 7	0 117	37 6
M NaCl	21 2	0 114	34 5
4M NaCl	19 1	0 110	38 0
4M Urea	26 6	0 174	43 8
6M Urea	27 3	0 201	48 9
8M Urea	30 8	0 198	45 2
11 9 Ethanol–water	24 0	0 108	31 4
6M Urea in 11 9 ethanol–water	24 4	0 137	41 8

From Table II, it may be seen that there is an increase in [η] and $(\overline{R_g^2})_z^{\frac{1}{2}}$ in the levan in water with rise in temperature and with addition of urea to the water and ethanol–water solvents Similarly both parameters are lower in ethanol–water than in pure water However the intrinsic viscosity is lower in 0 1M sodium chloride and decreases further at higher concentrations of salt whereas the $(\overline{R_g^2})_z^{\frac{1}{2}}$ values remain virtually constant, within experimental error

The results confirm previous findings[8 15] that, in solutions of native levan, it is unlikely that aggregates are formed by noncovalent, secondary forces between individual polymer molecules, as, otherwise, large decreases in molecular weights and sizes would be expected in some of the solvents This conclusion is in accord with the work of Antonini and co-workers[16] and Burchard and Pfannemüller[17], who found aggregation to be absent from solutions of dextrans having molecular weights $< \sim 100 \times 10^6$, even though the latter authors observed aggregation at higher molecular weights

The results also show that the $(\overline{R_g^2})_z^{\frac{1}{2}}$ of the polysaccharide may increase or decrease in the various solutions, indicating different polymer–solvent interactions and some coil flexibility

The polymer expands in water at 57°, as expected, as well as in urea solutions Urea is a good hydrogen-bonding agent, and may break intramolecular hydrogen-bonds, *e g*, between branches, which would allow the molecule to expand[18]

Contraction of the polymer coil in ethanol–water, as indicated by a decrease in $(\overline{R_g^2})^{\frac{1}{2}}$ and $[\eta]$, was anticipated, as ethanol is a precipitant for levan, and 11 9 ethanol–water at 25° is[6] a theta solvent The decrease in $[\eta]$ of levan in aqueous solutions of sodium chloride indicates that these are poorer solvents than water The observation that the radii of gyration did not decrease in salt solutions, where as the value of $[\eta]$ did, may be attributed to the conditions of the light-scattering experiments, which were performed on solutions that were two orders of magnitude more dilute than those used for measuring the viscosity, 100 μg mL^{-1} *versus* 10 mg mL^{-1} The light-scattering and viscosity results of Antonini *et al* [16] on dextran in water and in salt solutions exhibit a similar phenomenon Apparently, these polysaccharides of high molecular weight behave differently in dilute and concentrated solutions of salt

Although the levan macromolecule exists as a highly branched, compact coil in solution, it is, perhaps not surprising that it should exhibit substantial changes in size in the various solvents Interestingly, for cellulose, in addition to long-range interactions that affect the size of the coil, short-range, specific, solvent interactions may alter the angle at the glycosidic oxygen-atoms, and this may introduce large changes in molecular dimensions These conformational effects have been calculated[19] for cellulose

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