Carbohydrate Research, 108 (1982) 1–11
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

ASSESSMENT OF BRANCHING IN HYDROLYSATES OF *S. salivarius* LEVAN AND *L. mesenteroides* DEXTRAN FROM SMALL-ANGLE X-RAY SCATTERING

BEHROOZ A. KHORRAMIAN* AND SALVATORE S. STIVALA**
Department of Chemistry and Chemical Engineering, Stevens Institute of Technology, Hoboken, New Jersey 07030 (U.S.A.)

(Received December 24th, 1981; accepted for publication, February 23rd, 1982)

ABSTRACT

A fraction of low molecular weight from a partially acid-hydrolyzed *S. salivarius* levan was examined in aqueous solution at 25° by the method of small-angle X-ray scattering (SAXS). The weight-average molecular weight of 26,570 and radius of gyration of 47.6 Å are in good agreement with the values obtained previously from sedimentation equilibrium and light scattering, respectively. Although this fraction behaves hydrodynamically as a linear random coil, the higher mass per unit length $[(m_u)_b]$ of 45.82 daltons/Å (obtained from SAXS), compared with the calculated value of $(m_u)_l = 28.94$ for linear levan, confirms its branched structure. The *g*-factor, a measure of branching, was computed as 0.63 from the derived expression, $g = (m_u)_l/(m_u)_b = L/L_l$, where L and L_l are the hydrodynamic length (stretched length) of the backbone of the branched levan and linear levan, respectively. The values for persistence length ($a^* = 17.7$ Å), radius of gyration of the cross-section ($R_q = 8.2$ Å), and $L = 534$ Å were also obtained for the levan hydrolysate. The lower values of $(m_u)_b = 35.1$ Å and $R_q = 2.3$ Å, and the higher value of $g = 0.90$ for the *L. mesenteroides* dextran hydrolysate of comparable molecular weight, show that the levan hydrolysate is more branched. The higher value of $a^* = 26.2$ Å for the dextran suggests that its molecules are stiffer than those of the levan.

INTRODUCTION

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

*Present address; Department of Chemistry, Long Island University, Brooklyn, New York 11201, U.S.A.

**To whom reprint requests should be addressed.

The elution fractionation, chemical analysis, and intrinsic viscosities of *Streptococcus salivarius* levan in water and in dimethyl sulfoxide have been reported[5]. Various solution parameters obtained from light scattering and sedimentation analysis showed that the molecular weights (M) of the fractions varied[6,7] from $\sim 18 \times 10^6$ to 60×10^6. The *S. salivarius* levans used in these studies were highly branched, compact structures of near spherical symmetry in water. The molecular weight of the levan produced by *S. salivarius* may be related to the pH of the medium[8]. Light scattering and intrinsic viscosities showed that *S. salivarius* levan is not aggregated in water[9]. Kinetic studies[10,11] of the acid hydrolysis of levan demonstrated that the degradation may be non-random.

Garg and Stivala[12] recently reported on the longitudinal and cross-sectional properties of dextran in water from small angle X-ray scattering (SAXS). The radius of gyration (R_g), the radius of gyration of the cross-section (R_q), persistence length (a^*), and mass per unit length (m_u) were reported for various samples of hydrolyzed, fractionated *L. mesenteroides* dextran varying in M from 11,200 to 253,000. We now compare the aqueous solution parameters from SAXS of a fraction of low molecular weight obtained from partially hydrolyzed *S. salivarius* levan with those of the previously reported[13], unhydrolyzed levan of high molecular weight. Fractionated polymers from acid-hydrolyzed *S. salivarius* levans of $M > 10^5$ behave[14] as spheres in water, whereas those of $M < 10^5$ behave as linear random coils. Therefore, in view of this observation, the m_u and the branching parameter of the high *vs.* low levans were assessed.

EXPERIMENTAL

Material. — The partially acid-hydrolyzed levan used in this study was fraction E-7, prepared and characterized previously[14]. The weight-average molecular weight ($\overline{M}_w$) and the partial specific volume ($\bar{v}$) were reported as 2.73×10^4 and 0.65 $mL.g^{-1}$, respectively.

Method. — The procedure used in this work has been described previously[12]. Detailed procedures, theory, and treatment of data may be found elsewhere[15,16]. An entrance slit of 150 μm and counter-slit of 375 μm were used in the Kratky camera. The collimation error of the raw data was eliminated on a PDP-10 computer, according to the method of Glatter[17]. The corresponding, corrected data are the desmeared scattering data.

Measurements of the levan in water were made at $25 \pm 0.2°$ for six concentrations ranging from 20.8 to 42.3 $mg.mL^{-1}$. Concentration dependence of the data was eliminated by extrapolation to infinite dilution.

The molecular weight (M) was obtained according to Eq. *1*.

$$M = (I_0/P_0)\, 21.0a^2/(z_1 - \bar{v}_1\rho_2)^2\, dc, \qquad (1)$$

where I_0 is the scattered intensity extrapolated to zero-scattering angle, a is the distance in cm between the sample and plane of registration, d is the sample thickness

($\sim$0.1 cm), c is the concentration in g.mL^{-1}, $\bar{v}$ is the partial specific volume of the solute, ρ_2 is the number of mol of electrons per mL of the solvent (0.555 for water), z_1 is the number of mol of electrons per g of the solute (0.5304 for levan based on its structural formula), and P_0 is the energy of the primary beam per cm length in the plane of registration. The constant 21.0 is equal to $1/I_e N_A$, where I_e is the scattering power of one electron (7.9×10^{-26}, Thomson's constant) and N_A is Avogadro's number. The concentration dependency of M was eliminated[15] by extrapolating the I/c curves to $c = 0$.

The radius of gyration of the levan was obtained from the Guinier equation,

$$\ln I = \ln I_0 - K R_g^2 (2\theta)^2, \tag{2}$$

where $K = (2\pi/\lambda)^2/3$, or

$$I = I_0 \exp(-R_g^2 Q^2/3), \tag{2a}$$

where $Q = (4\pi/\lambda)\sin\theta$ [but at very low angle $Q \approx (4\pi/\lambda)\,\theta \approx (2\pi/\lambda)\,(2\theta)$] (for $\lambda =$ 1.54 Å, $K = 5.5488$) and 2θ is the scattering angle in radians. Since decay of the intensity at very small angles (inner portion of the scattering curve) is a Gaussian function of 2θ, a straight line is obtained for the several innermost points according to Eq. *2* or *2a*. The slope of this linear portion yields the radius of gyration, and the intercept yields the intensity at zero angle. Since R_g is concentration dependent, the apparent values of R_g for each concentration, obtained from the Guinier plots, are plotted against c to obtain the value of R_g at infinite dilution. This value would be the root-mean-square value, $(\overline{R_g^2})^{\frac{1}{2}}$, since the fractionated levan is polydisperse, however low.

The radius of gyration of the cross-section was obtained from the equations

$$\ln (I2\theta) = \ln (I2\theta)_0 - K' R_q^2 (2\theta)^2, \tag{3}$$

or

$$IQ = (IQ)_0 \exp(-R_q^2 Q^2/2), \tag{3a}$$

where $K' = (2\pi/\lambda)^2/2$ (for $\lambda = 1.54$ Å, $K' = 8.3232$). The slope of Eq. *3a* yields R_q. The values of R_q obtained from the slopes at various concentrations were plotted against c, from which R_q at infinite dilution was obtained.

The mass per unit length is calculated from the absolute value of the intensity at zero angle of the cross-section factor, $(I2\theta)_0$, using an equation similar to Eq. *1*, namely,

$$m_u = [(I2\theta)_0/P_0][27.3\, a^2/(z_1 - \bar{v}_1 \rho_2)^2 dc], \tag{4}$$

where 27.3 is a constant equal to $2/(\lambda I_e N_A)$. The intercepts of the linear slots of Eq. *3* or Eq. *3a* are the values of $(2\theta I)_0$ for the various concentrations. The plot of $\ln (I2\theta)/c$, or $\ln (IQ)_0/c$, *vs.* c yields the value of $(I2\theta/c)_{c\to 0}$. This value and Eq. *4* yield the mass per unit length.

The persistence length was calculated from the equations:

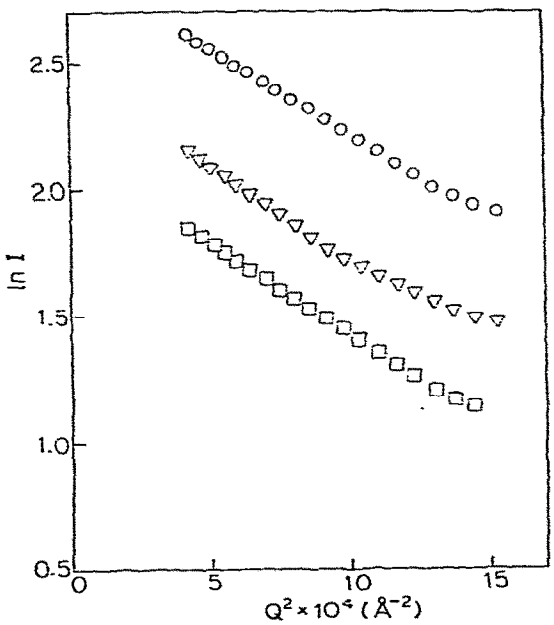

Fig. 1. Guinier plot of levan in water at 25° for three concentrations: ○, 42.3 mg.mL^{-1}; △, 39.3 mg.mL^{-1}; and □, 35.6 mg.mL^{-1}.

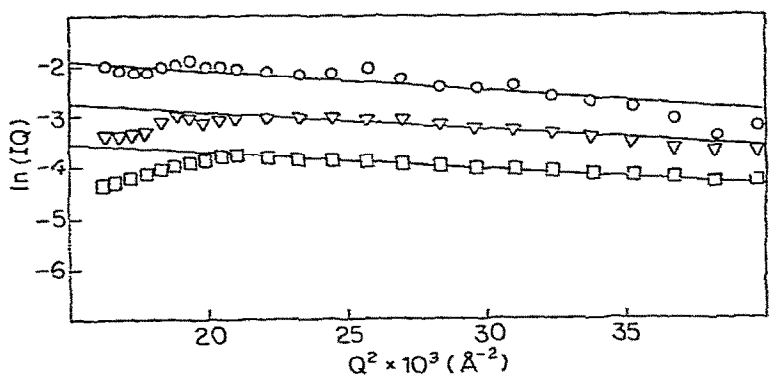

Fig. 2. Plot of ln (IQ) *versus* Q^2 for levan in water at 25° at three concentrations: ○, 42.3 mg.mL^{-1}; △, 35.6 mg.mL^{-1}; and □, 25.0 mg.mL^{-1}.

$$a^* = (2)\,(2.3)\,(\lambda/4\pi)/2\theta)^* \tag{5}$$

or

$$a^* = 2.3/Q^*, \tag{5a}$$

where $(2\theta)^*$ is the transition between the $1/(2\theta)^2$ and $1/(2\theta)$ portions of the scattering curve. The plot of (IQ^2) *vs.* Q for the sample yields the value of Q^*. The value of a^* is obtained from Eq. *5a* and Q^* (the transition is indicated by the vertical line in Fig. 6).

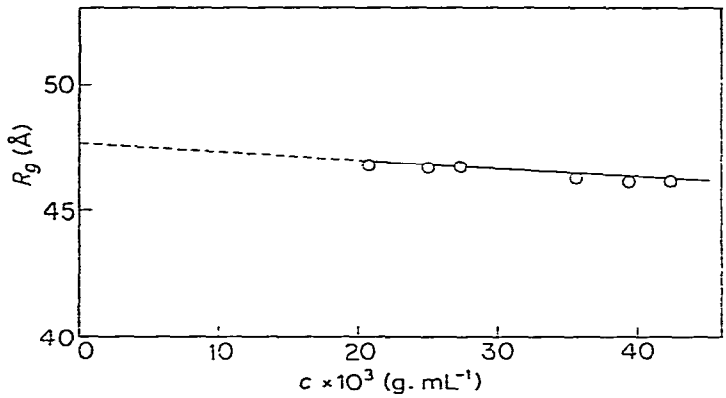

Fig. 3. Radius of gyration (R_g) of levan as a function of concentration in water at 25°.

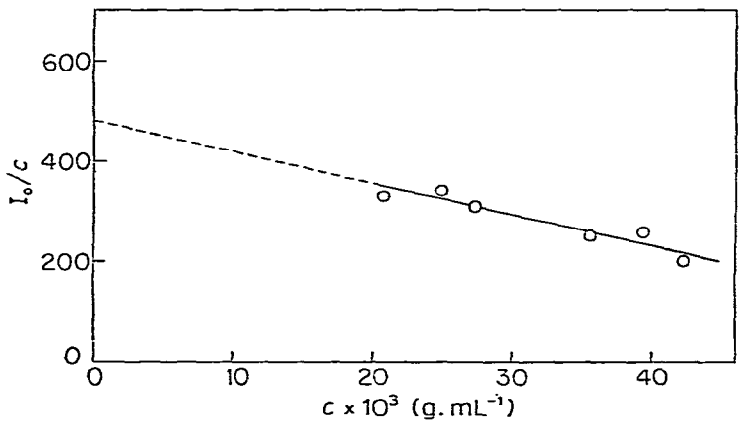

Fig. 4. Plot of I_0/c *versus* c for levan in water at 25°.

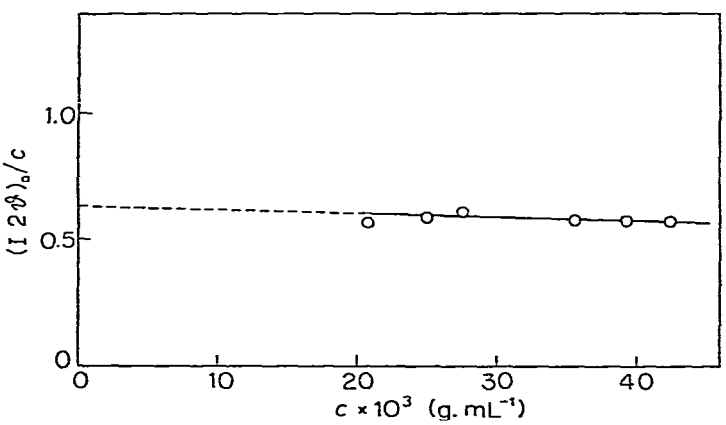

Fig. 5. Plot of $(I2\theta)_0/c$ *versus* c for levan in water at 25°.

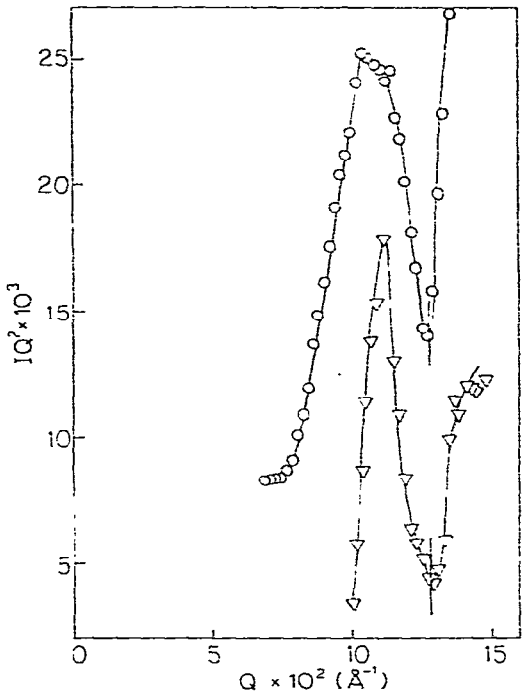

Fig. 6. Plot of (IQ^2) *versus* Q for levan in water at 25° at two concentrations: ○, 42.3 mg.mL^{-1}; and △, 27.3 mg.mL^{-1}.

RESULTS

Fig. 1 shows the Guinier plot, according to Eq. *2a*, of the levan E-7 for one given concentration, from which the radius of gyration (R_g) is obtained. Fig. 2 shows the plot of the cross-section according to Eq. *3a*, from which were obtained values for the radii of gyration of the cross-section (R_q). The concentration dependencies of R_g, I_0/c, and R_q were eliminated by extrapolation of the data to infinite dilution. Figs. 3 and 4 show plots of R_g and I_0/c *vs.* c, respectively. Table I contains the values of R_g, I_0/c, and R_q at infinite dilution.

Fig. 5 is the plot of $\ln(2\theta I)_0/c$, from which the value for $(2\theta I/c)_{c\to 0}$ of 0.6288 was obtained. The mass per unit length (m_u) of the levan E-7 was calculated from this value using Eq. *4* (see Table I).

Fig. 6 is the plot of (IQ^2) *vs.* Q for the levan hydrolysate, from which the value of 0.13 per Å for Q* was obtained. The value of the persistence length (a^*) was calculated from this value and Eq. *5a* (see Table I).

Table I summarizes the weight-average molecular weight ($\overline{M}_w$) and SAXS data for the levan hydrolysate E-7. Included in Table I are the SAXS data for a fractionated sample of native *S. salivarius* levan[13] (F-14) and two *L. mesenteroides* dextran hydrolysates[12] from the previous studies, for comparison.

The values of $\overline{M}_w$ = 26,570 and R_g = 47.6 Å for the levan fraction E-7 (*cf.*

TABLE I

SAXS DATA FOR LEVAN AND DEXTRAN IN H_2O AT 25°

Parameter	*Levan*		*Dextran*[b]		
	Hydrolysate E-7	*Native*[a] *F-14*	*T-40*	*T-10*	*T-average*
$\bar{M}_w$	26,750	16.9×10^6	42,000	12,000	27,000
R_g (Å)	47.6	395	68	38	53
R_q (Å)	8.2	22	2.65	2.00	2.3
m_u (daltons/Å)	45.82	72.9	35.7	34.5	35.1
a^* (Å)	17.7	42.8	24.8	27.5	26.2
L (Å)	534	2.30×10^5	1,110	355	733
g	0.63	0.39	0.88	0.91	0.90

[a]Ref. 13. [b]Ref. 12.

Table I), obtained from SAXS in this work, were in good agreement with the previously reported[13] values of $\overline{M}_w = 27{,}300$ from sedimentation equilibrium, and $R_g = 42.8$ Å calculated from intrinsic viscosity and $\overline{M}_w$. The values of R_g and R_q, m_u, and a^* for E-7 are lower than those for the native levan (F-14) of much higher molecular weight ($\overline{M}_w = 16.9 \times 10^6$) (*cf.* Table I). The smaller difference between the corresponding parameters for the two dextran hydrolysates, T-40 and T-10, reflect the smaller differences in their molecular weights. The M of these dextrans are of the same order of magnitude as that for the E-7 levan (for purposes of comparison, the last column in Table I lists the average values of the parameters for T-40 and T-10 that a dextran of $\overline{M}_w = 27{,}000$ may be expected to have). In light of this, it is noted from Table I that the radius of gyration of the cross-section (R_q) is about four times higher for the E-7 levan than for the dextran having approximately the same M, whereas the persistence length (a^*) for the dextran is higher than for the E-7 levan. The latter suggests that the levan is less stiff than the dextran of comparable M.

DISCUSSION

The hydrodynamic or total length of the molecule (stretched length) may be obtained from the division of Eq. *1* by Eq. *4*, thus,

$$L = M/m_u = 0.769\,[(I_0/c)/(I2\theta)_0 c]_{c\to 0} \tag{6}$$

or

$$L = \lim_{c\to 0} 0.769\, I_0/(I2\theta)_0. \tag{6a}$$

The value of L obtained for levan E-7 is shown in Table I, and is taken as the longest length, or backbone, of the molecule[21,22].

The hydrodynamic or total length, L_l, of the linear polymer may be obtained from

$$L_l = (M_l/M_0)h, \tag{7}$$

where M_0 and h are the molecular weight and length of the monomer unit, respectively (the subscript l refers to linear). The mass per unit length $[(m_u)_l]$ for the linear polymer may be obtained from

$$(m_u)_l = M_l/L_l = M_0/h. \tag{8}$$

The length (h) of 2,6-anhydro-β-D-fructofuranose from X-ray diffraction was reported[18] as 5.874 Å. Neutron scattering[19] and X-ray diffraction[20] yielded the values of h for sucrose of 10.8633 Å and 10.8648 Å, respectively. We calculated, from bond angles and bond lengths, a value of h of 5.344 Å for the β-D-fructofuranosyl residue of sucrose. Using the averaged values of h of 5.874 Å and 5.344 Å (h = 5.609 Å) and M_0 = 162 for the fructofuranosyl residue in Eq. *8*, $(m_u)_l$ = 28.94 daltons/Å. The higher value for m_u (45.82 daltons/Å, *cf.* Table I) for the levan E-7 in this study indicates that E-7 is branched.

Branched molecules are more compact than linear molecules of the same molecular weight and chemical composition. Therefore, the density of the polymer segments near the center of mass of the branched polymer is higher than for its linear counterpart. Accordingly, the radius of gyration of the branched polymer $[(R_g)_b]$ is less than the radius of gyration of the linear polymer $[(R_g)_l]$ in the same solvent. The g-factor, defined by Zimm and Stockmayer[23] as Eq. *9*, is taken as a measure of branching.

$$g = (R_g)_b^2/(R_g)_l^2 \tag{9}$$

The value of g decreases rapidly as the number of branches increases. Orofino[24] (Eq. *10*) and Casassa and Berry[25] (Eq. *11*) expressed the g-factor in terms of the weight fraction of the backbone (λ) and number of branches (f) for comb-branched polymers.

$$g = \lambda + (1 - \lambda)f^{-2} + 3(1 - \lambda)^2 f^{-1} - 3(1 - \lambda)^2 f^{-2} \tag{10}$$

$$g = \lambda + (1 - \lambda)^{7/3}(3f - 2)f^{-2} \tag{11}$$

The g-factor expressed by Eqs. *10* or *11* approaches the value of λ for very large values of f. Hence,

$$g \approx \lambda = M_{bb}/M, \tag{12}$$

where M_{bb} is the molecular weight of the backbone, equal to $(L/h)\,M_0$ (and thus M_{bb} = 15,539), and M is the molecular weight of the branched polymer. Seymour[26] reported from ^{13}C-n.m.r. data that, in levan, the ratio of branched monomer to non-branched monomer is 1 to 7, *i.e.*, a branch point occurs at every eighth monomer. Based on this estimate, the number of branch points on the levan backbone is obtained from its hydrodynamic length (L) and h_{av} = 5.609 Å for the β-D-fructofurano-

syl residue, *i.e.*, $f = (L/h)/8$. Based on this calculation, the number of branch points on the levan backbone (f) was estimated as 5.165×10^3 for the native levan[13] F-14 ($M_{bb} = 6.64 \times 10^6$); for the levan E-7, in this study, $f = 12$. Therefore, $g = 0.39$ for levan F-14 estimated from Eq. *12*; for E-7, $g = 0.63$ calculated from Eq. *10* (or $g = 0.59$ estimated from Eq. *12*). It should be mentioned, however, that levan is dendritic (branched branch), and, therefore, the estimated values of f are the number of dendritic branches attached to the linear backbone of length L. It is implicit in the g-factor expressed by Eq. *10* or Eq. *11* that, in comb-like polymers, the branches attached to the backbone are not branched. Hence, it is reasonable to assert that, for dendritic structures or highly branched structures, the weight-fraction of the backbone (λ), as expressed by Eq. *12*, is a good estimate of branching. In this case, SAXS can readily provide the $(m_u)_b$ from which L is calculated from Eq. *6*. The M_{bb} value can be estimated from L, and from M_0 and h of the monomer unit. The latter may be available from X-ray diffraction or neutron scattering, or may be calculated from bond lengths and bond angles.

The mass per unit length of the branched polymer $[(m_u)_b]$ may be expressed as

$$(m_u)_b = M/L, \tag{13}$$

where L is the hydrodynamic or total length of the backbone and M is the molecular weight of the branched polymer. The M_{bb} of the linear backbone may be expressed in terms of its m_u and its L as

$$M_{bb} = (m_u)_l L. \tag{14}$$

It follows, from Eqs. *13* and *14*, that Eq. *12* may be written as

$$g \approx \lambda = (m_u)_l/(m_u)_b. \tag{12a}$$

Further, for a branched polymer and its linear counterpart having the same M, it follows (see ref. 13) from Eqs. *8* and *13* that

$$(m_u)_l/(m_u)_b = L_l/L = \lambda. \tag{15}$$

To obtain the g-factor, as expressed by Eq. *9*, requires the availability of the linear polymer having the same M and chemical composition as the branched polymer. Further, the R_g for both the branched and linear polymers should be obtained in the same solvent at the same temperature, in this case, the theta temperature (T_θ). It is often not possible to obtain both linear and branched polymers having the same M, nor a linear polymer of any molecular weight. In any event, determination of T_θ is not only tedious but its value is often different[27] for branched and linear structures having the same M and chemical composition. The g-factor computed from R_g values reflects the spatial size, in a given solvent at a given temperature, of the branched structure to its linear counterpart. Had the R_g for the linear levans been available, the values of g would probably have been higher than the g values computed from λ.

In the absence of branching, *i.e.*, for the linear structure, the $(m_u)_l$ for dextran and levan are 31.46 and 28.94 daltons/Å, respectively, calculated from Eq. *8*. It would then follow for branched dextran and levan having the same M, and where the number and nature of branches are identical in both, that $(m_u)_b$ would be higher for the dextran. However, m_u for levan E-7 is higher than for dextran of comparable M (*cf.* Table I, T-Average). This suggests that levan E-7 has less but heavier branches on the backbone. This difference is also indicated from the lower *g*-value for levan E-7, and explains the higher radius of gyration of the cross-section (R_q) for levan E-7, as may be noted from Table I.

From viscosity, sedimentation, and light-scattering data, Stivala and Zweig[14] found that *S. salivarius* levan fractions of $M > 10^5$ behave as spheres and those of $M < 10^5$ behave as random coils in water at 25°. The ^{13}C-n.m.r. spectra for some of the fractions of the latter were identical with those of the branched structures of the former. That the levan fraction E-7 (one of the fractions of the latter) is indeed branched was confirmed from its m_u (*cf.* Table I), thus confirming the inference based on ^{13}C-n.m.r. data. The viscoelastic and/or solution properties of polymers are profoundly affected by a few long branches (even by one or two), but not by many short branches. Hence, the linear random-coil behavior of levan fractions of $M < 10^5$ may be explained on the basis that their branches, though many, are relatively short. The R_g value of 42.8 Å for fraction E-7, reported by Stivala and Zweig[14], was calculated from its intrinsic viscosity and molecular weight, using the Flory–Fox theory for linear random coils. This value is in very good agreement with the value of 47.6 Å for E-7 obtained experimentally from SAXS (*cf.* Table I).

Finally, it should be mentioned that the parameters obtained for the branched levan are based on plots of experimentally determined SAXS data customarily used for linear polymers. However, observations made on dextran fractions and model branched-polystyrenes, previously thoroughly characterized from solution by other various methods[12,21,22], demonstrate that the method is also applicable to branched polymers. Further, Garg and Stivala[12] constructed a linear model for dextran from the SAXS data for the branched dextran. They found absolute compatibility, and the *g*-factors calculated from various theories and from Eq. *12* were all in excellent agreement. That the total length (L) from Eq. *6* is the longest length, and hence the backbone, was demonstrated from model comb-branch and star polystyrenes[21,22], of known values of L.

The recent work of Marshall and Weigel[28] on the enzymic degradation of the levan elaborated by *S. salivarius* strain 51 is noteworthy. These workers demonstrated by linkage analysis that the levan is composed of multiply branched chains. The work of Marshall and Weigel demonstrates the detection of branching in *S. salivarius* levan by chemical methods, compared with the physical detection of branching by SAXS described in this paper.

ACKNOWLEDGMENTS

This work was supported, in part, by a grant from the National Science Foundation DMR-7724634 and funds from the Rene Wasserman Foundation.

REFERENCES

1 H. Hibbert and R. S. Tipson, *J. Am. Chem. Soc.*, 52 (1930) 2582; R. L. Whistler and C. L. Smart, *Polysaccharide Chemistry*, Academic Press, New York, 1958.
2 C. T. Greenwood, *Adv. Carbohydr. Chem.*, 7 (1952) 289–332.
3 C. F. Niven, Jr., K. L. Smiley, and J. M. Sherman, *J. Bacteriol.*, 41 (1941) 479–484.
4 S. A. Leach, in W. D. McHugh (Ed.), *Dental Plaque*, Thomson, Dundee, Scotland, 1970, p. 143.
5 J. Ehrlich, S. S. Stivala, W. S. Bahary, S. K. Garg, L. W. Long, and E. Newbrun, *J. Dent. Res.*, 54 (1975) 290–295.
6 S. S. Stivala, W. S. Bahary, L. W. Long, J. Ehrlich, and E. Newbrun, *Biopolymers*, 14 (1975) 1283–1292.
7 W. S. Bahary, S. S. Stivala, E. Newbrun, and J. Ehrlich, *Biopolymers*, 14 (1975) 2467–2478.
8 L. W. Long, S. S. Stivala, and J. Ehrlich, *Arch. Oral Biol.*, 20 (1975) 503–507.
9 S. S. Stivala and W. S. Bahary, *Carbohydr. Res.*, 67 (1978) 17–21.
10 M. D. Lauren, S. S. Stivala, W. S. Bahary, and L. W. Long, *Biopolymers*, 14 (1975) 2373–2385.
11 S. S. Stivala, M. D. Lauren, and S. K. Garg, *Polymer*, 20 (1979) 18–22.
12 S. K. Garg and S. S. Stivala, *J. Polym. Sci., Polym. Phys. Ed.*, 16 (1978) 1419–1434.
13 S. S. Stivala and B. A. Khorramian, *Carbohydr. Res.*, 101 (1982) 1–11.
14 S. S. Stivala and J. Zweig, *Biopolymers*, 20 (1981) 605–619.
15 S. S. Stivala, M. Herbst, O. Kratky, and I. Pilz, *Arch. Biochem. Biophys.*, 127 (1968) 795–802.
16 I. Pilz, O. Glatter, and O. Kratky, *Methods Enzymol.*, 61 (1979) 148–249.
17 O. Glatter, *J. Appl. Crystallogr.*, 7 (1974) 147–153.
18 J. C. Hanson, L. C. Sieker, and L. H. Jensen, *Acta Crystallogr., Sect. B*, 29 (1973) 797–808.
19 G. M. Brown and H. A. Levy, *Acta Crystallogr., Sect. B*, 29 (1973) 790–797.
20 W. Dreissig and P. Luger, *Acta Crystallogr., Sect. B*, 29 (1973) 1409–1416.
21 S. K. Garg and S. S. Stivala, *Polymer*, 23 (1982) 514–520.
22 B. A. Khorramian, Ph.D. Dissertation, Stevens Institute of Technology, Hoboken, N.J., 1980.
23 B. H. Zimm and W. H. Stockmayer, *J. Chem. Phys.*, 17 (1949) 1301–1314.
24 T. A. Orofino, *Polymer*, 2 (1961) 295, 305–314.
25 E. F. Casassa and G. C. Berry, *J. Polym. Sci., Part A*, 4 (1966) 881–897.
26 F. R. Seymour, R. D. Knapp, and A. Jeanes, *Carbohydr. Res.*, 72 (1979) 222–228.
27 F. Candau, P. Rempp, and H. Benoit, *Macromolecules*, 5 (1972) 627–635.
28 K. Marshall and H. Weigel, *Carbohydr. Res.*, 83 (1980) 321–326.