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MOLECULAR WEIGHT DISTRIBUTION AND BRANCHING FREQUENCY OF DEXTRAN PRODUCED BY FREE AND IN CALCIUM ALGINATE IMMOBILIZED LEUCONOSTOC MESENTEROIDES

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ABSTRACT

Dextran was produced using in 3% calcium alginate immobilized and free cells of L. mesenteroides ATCC 10830 (NRRL B-512 F). Information about the molecular structure of the produced polymers using size exclusion chromatography and on-line low angle laser light scattering (SEC/LALLS) detection was obtained. Dextran formed outside the beads by immobilized cells possesses an average molecular weight of about 1.84×10^7 daltons. Average molecular weight of dextran formed by free cells, however, was 1.32 times (2.43×10^7 daltons for free cells) that formed by immobilized cells. Determination of the qualitative branching parameters (G^{-1}_V and G_M) of dextran formed by both cultures shows that branching of dextran produced by free cells was also higher than that produced by immobilized cells in calcium alginate.

INTRODUCTION

Dextransucrase (sucrose 1,6-α-D-glucan-6-α-glucosyl transferase EC 2.4.1.5) is an extracellular enzyme or a group of enzymes responsible for the synthesis of dextran (glucan) from sucrose[1]. Dextran is a branched polymer in which the linear parts result from the linear condensation of D-glucopyranosyl units in α-D-(1----> 6) glucosidic bonds, whereas branching points result from α-D(1 -----> 3), α-D-(1 ----> 2) or α-D-(1 -----> 4) glucosidic bonds[2]. The degree of branching [measured as non-(1 ----> 6) bonds] affects the water solubility and rheological properties of the dextran polymer. L. mesenteroides ATCC 10830 (NRRL B-512F) produces a low branched dextran polymer containing about 95% α-D-(1 ----> 6) bonds and 5% α-D-(1 ----> 3), bonds, and which is highly soluble in water. The side chains of the dextran obtained from this bacterium contain 2 or more glucose residues attached by α-D-(1 ----->6) bonds[3].

Size exclusion chromatography (SEC) is regarded as one of the most important analytical techniques in polymer characterization[4-6]. With the development of on-line low-angle laser light scattering (LALLS) detection in the late 1970s, molecular weights, molecular weight distributions, and polymer branching can now be rapidly determined as a function of polymer molecular size. On-line light scattering detection provides a simple and direct calibration method. The combination of SEC molecular weight detection, and microcomputer data acquisition provides an efficient

method of determining molecular weight and long-chain branching of water-soluble polymers[5,7-10].

This paper deals with the molecular characterization of dextran produced by in calcium alginate beads immobilized and free cells of L. mesenteroides ATCC 10830 (NRRL B-512F) as detected by size exclusion chromatography and on-line low-angle laser light scattering.

MATERIALS AND METHODS

Microorganism and Fermentation Conditions:

The dextran-producing strain L. mesenteroides ATCC 10830 (NRRL B-512F) was immobilized in 3% calcium alginate beads and a fed-batch dextransucrase (DS) production cycle for 24 hours was performed. Then, the sucrose concentration was raised to 10% (w/v), pH was adjusted to 5.2 ± 0.1, temperature was controlled at 30°C and a dextran production cycle by the immobilized cells was performed for 24 hours[11-13]. Dextran production by free cells was performed under similar conditions as immobilized cells[11-13]. Fermentations were performed in a 1L-bubble column containing 200 g of beads and 800 ml of medium[11,12]. After fermentation, the culture broth was centrifuged at 4000 xg at 4°C for 10 minutes and the dextran in the supernatant was recovered by precipitation with 2 volumes of ethanol. The precipitated dextran was purified 3-4 times by dissolving in sterile demineralized water and reprecipitation with 2 volumes of ethanol. Purified dextran was dried under vacuum at room temperature.

Size Exclusion Chromatography and On-Line Low-Angle Laser Light Scattering (SEC-LALLS) Detection:

Theoretical background of the analysis, instruments, columns and elution system were the same as described previously[9,10]. In short, the SEC/LALLS system consisted of a pump (M6000, Waters Associates, Milford, MA), injector (U6K) and differential refractive index (DRI R401), LDC/Milton Roy (Riviera Beach, Florida) KMX-6 LALLS detector, and a TSK (40S and 65F) Fractogel HW columns. The LALLS photometer with a flow-through cell was serially conneceted with the DRI detector. Scattering intensity data were collected at the 6-7° annulus with 6328Å wavelength, He-Ne laser. Dextran T-500 (Pharmacia Fine Chemicals, Piscataway, NJ) was used as a standard linear polymer. Samples of test and standard dextran were prepared in degassed 0.5N NaOH aqueous solution, and the same solvent was used as the SEC eluent. The experiments were run with a flow rate of 0.2 ml/min at ambient temperature. Polysaccharide solutions were prepared for injection by dissolving known mass quantities of material and diluting to a known volume with the solvent. Both eluent and dextran solutions were filtered through a 0.2 µm Fluoropore filter (Millipore Corp). Just before LALLS cell. Values of specific refractive index increment (dn/dc) and the resultant LALLS optical constant K employed is listed for dextran as: dn/dc = 0.142 ml/g and K = 1.46E-7 mol cm^2/g^2.

RESULTS

1. Determination of Molecular Weight Distribution (MWD) of the Produced Dextran Polymers.

Figures 1A-C show the SEC/LALLS chromatograms collected from DRI and LALLS detectors for dextran T-500, dextran produced by immobilized and free cells of L. mesenteroides ATCC 10830 (NRRL B-512F), respectively. These figures give the intensity of responses measured in both LALLS and DRI cells as a function of retention time. From these figures, a fairly good separation was obtained via LALLS and DRI responses in the three analyzed types of dextran, especially through the LALLS. In the three analyzed dextrans, LALLS show only one peak in each case. The time of appearance of the peaks is inversely proportional to the molecular

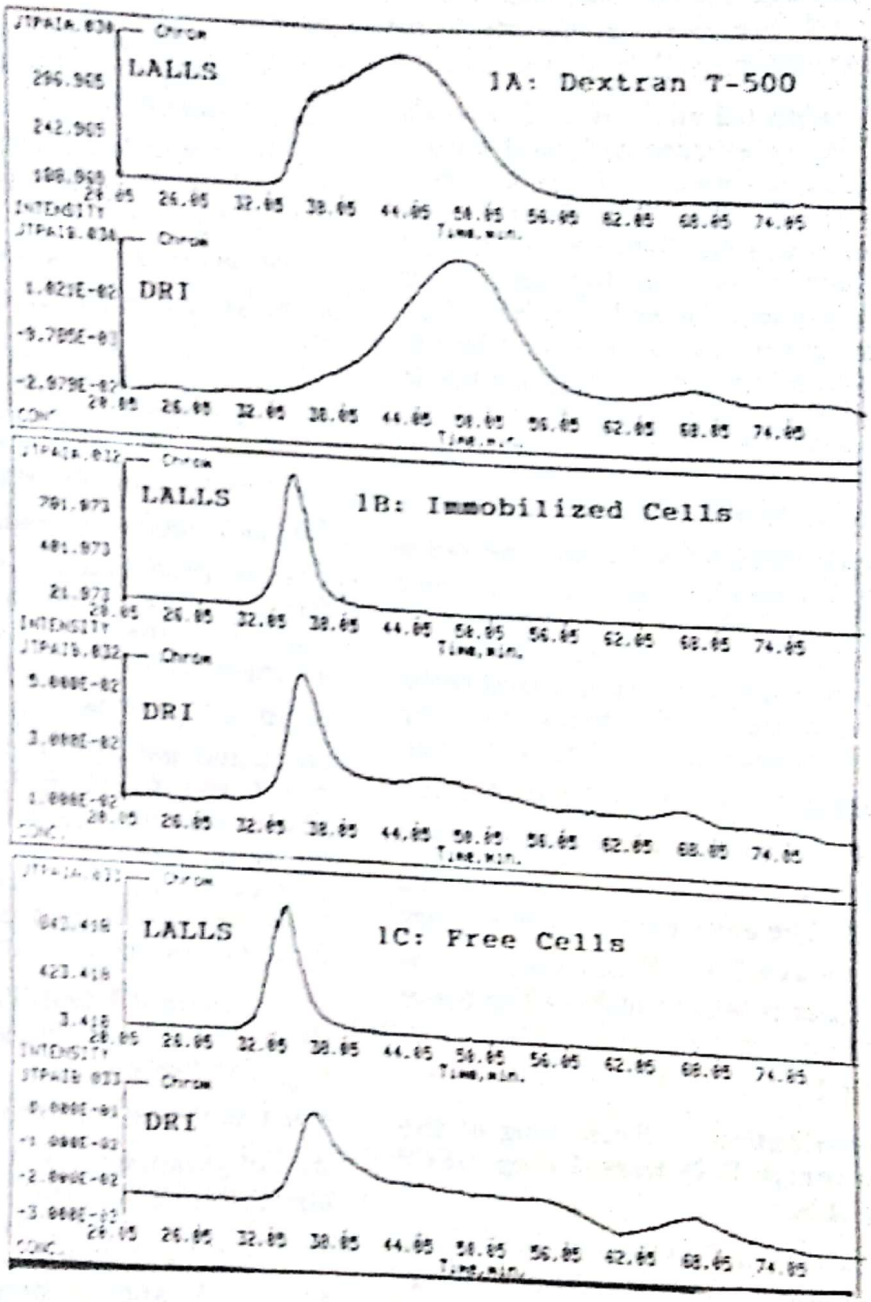

Figure (1): SEC/LALLS chromatograms as detected by differential refractive index (DRI) detector and low angle laser light scattering (LALLS) detector for dextran T-500 (A), and dextran produced by in calcium alginate immobilized (B) or free cells (C) of L. mesenteroides ATCC 10830 (NRRL B-512F).

weight of the separated polymer. The DRI detects also an early peak that almost corresponds to the peak detected by the LALLS, but minor elevations were also detectable in the three cases.

Through the analysis of 2-seconds data output, an average molecular weight via LALLS was measured and recorded. The differential weight fractions (molecular weight distribution, MWD) were plotted vs. the log molecular weights. Figures 2A and 2B show such relationship for dextran produced by immobilized and free cells during the whole course of analysis (total eluted volume in each case was about 15 ml), respectively. In case of dextran produced by immobilized cells, MWD is concentrated in a main peak around 5 x 10^7 daltons, but a wide left shoulder was also evident (Figure 2A). MWD of dextran produced by free cells behaves similarly to that produced by immobilized cells, but with 4 main differences: (1) The range of molecular weight distribution was narrower (between about 4 x 10^6 and 8 x 10^7 daltons compared to about 10^5 and 4 x 10^7 daltons for immobilized cells), (2) The earlier small peak appears latter (at about 7 x 10^6 daltons), (3) The left shoulder is bigger, and (4) The major peak is broader and has a maximum at about 7 x 10^7 daltons (Figure 2B).

2. Determination of Branching of the Produced Polymers Using SEC/LALLS.

SEC/LALLS system is also applicable for study of branched polysaccharides[9,10]. The Log M_w vs. elution volume (EV) of the standard dextran T-500 as well as the produced dextran by immobilized and free cells were analyzed by SEC/LALLS and are shown in Figure 3. Note that the data given in Figure 3 are limited to elution volumes between 7 and 11 ml (Flow rate was 0.2 ml/minute). Figure 3 indicates that a reverse linear relationship exists between Log M_w and EV for both dextran T-500 and dextran produced by immobilized and free cells, with an evident higher molecular weight at the same elution volume in the latter case.

As reported by Yu and Rollings (1987,1988)[9,10], the SEC/LALLS data can be transcribed into branching parameter values. Branching parameters are indicated by inverse of molecular wight ratio of linear vs. branched polymer (G^{-1}_V) at the same elution volume.

$$G_V = (M_l / M_b)$$

where the subscripts l and b denote linear and branched polymers, respectively. Using the standard dextran T-500 as a linear dextran, G^{-1}_V values of dextran produced by immobilized and free cells are given in Figure 4A and Table 1. In the range examind (EV = 7-11 ml at flow rate = 0.2 ml/min), the mean G^{-1}_V of dextran produced by immobilized and free cells are about 7.675 ± 0.8 and 41.21 ± 3.1, respectively. These values indicate that a dextran molecule produced by immobilized or free cells is approximately 7.7 or 41 times heavier than an equivalent hydrodynamic volume of dextran T-500.

Figure 4B and Table 1 give also the values of the branching parameter G_M. The branching parameter G_M is defined as the ratio of the mean-square radius of gyration (R^2) of branched and linear polymer of the same molecular weight ($G_M = R^2_b/R^2_l$ where the subscripts l and b denote linear and branched polymers, respectively). Radius of gyration is the end to end distance of the molecule. Yu and Rollings (1987)[9] used SEC/LALLS data values for determination of G_M applying the following equation:

$$G_M = G_V^{(a+1)/e}$$

Where a is the Mark-Houwink coeffi-

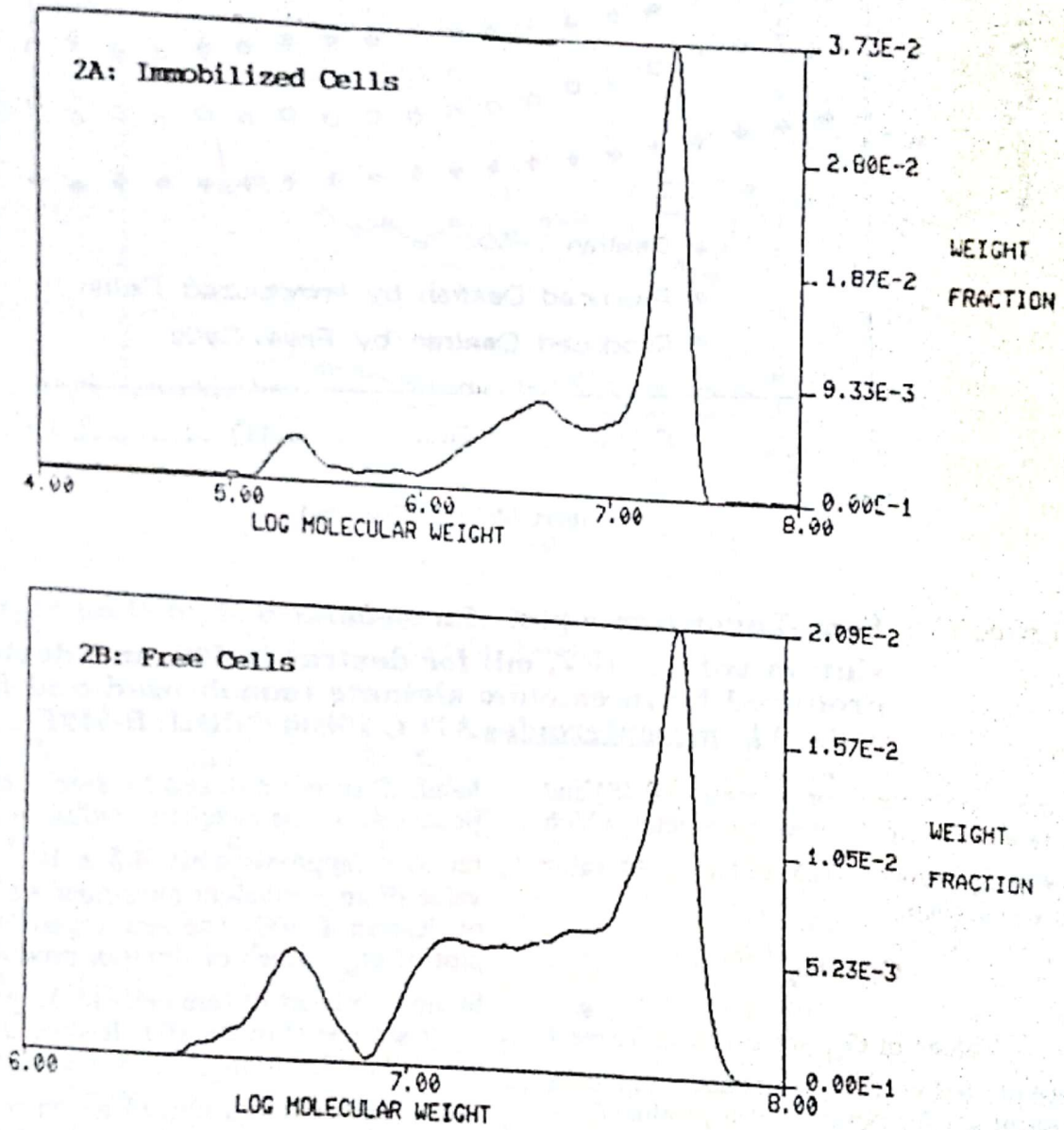

Figure (2): Differential weight fraction plot detected by SEC/LALLS for dextran produced by in calcium alginate immobilized (total injected mass = 69.13 µg) (A) and by free cells (total injected mass = 80.9 µg) (B) of L. mesenteroides ATCC 10830 (NRRL B-512F).

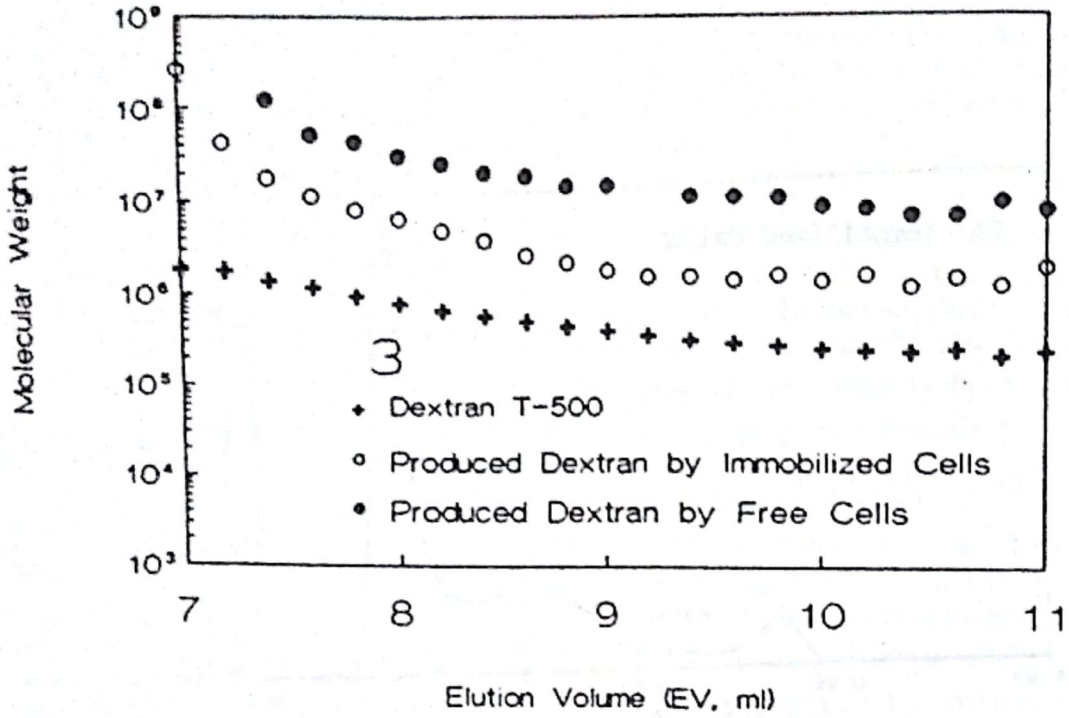

Figure (3): Semi-Logarithmic plot of molecular weight (Log M_W) vs. elution volume (EV, ml) for dextran T-500; and dextran produced by in calcium alginate immobilized and free cells of L. mesenteroides ATCC 10830 (NRRL B-512F).

cient (the value for dextran = 0.48) and e is a polymer draining parameter which can vary between 0.5 and 1[9]. At value of e = 1, then:

$$G_M = G_V^{(1.48)}$$

Values of G_M are given in Table 1 and plotted vs. Log. molecular weights in Figure 4B for both dextran produced by immobilized and free cells, respectively. Table 1 shows that the mean G_M value of dextran produced by the immobilized and free cells are in average $6.27 \times 10^{-2} \pm 6.02 \times 10^{-3}$ and $4.5 \times 10^{-3} \pm 2.8 \times 10^{-4}$, respectively. These values represent the ratios of branched (product) and linear (dextran T-500) mean-square radii of gyrations. Dextran produced by immobilized cells exhibits a mean-square radius of gyration approximately 0.06 the value of an equivalent molecular weight of dextran T-500 (Table 1). On the other hand, dextran produced by free cells possesses a mean-square radius of gyration of approximately 4.5×10^{-3} the value of an equivalent molecular weight of dextran T-500. The semilogarithmic plot of G_M values of dextran produced by immobilized or free cells looks principly similar (Figure 4B). It should be noted that the scale of plotting of G_M values of dextran produced by immobilized cells is 20 times the scale of G_M values of that produced by free cells vs. molecular weight.

DISCUSSION

Aqueous SEC/LALLS has been applied in determining information about molecular weight and branching frequencies of dextran produced by in calcium alginate immobilized and free cells of L. mesenteroides ATCC 10830 (NRRL B-512F). The presented data show that some structural differences exist between dextran formed in the cell-free culture

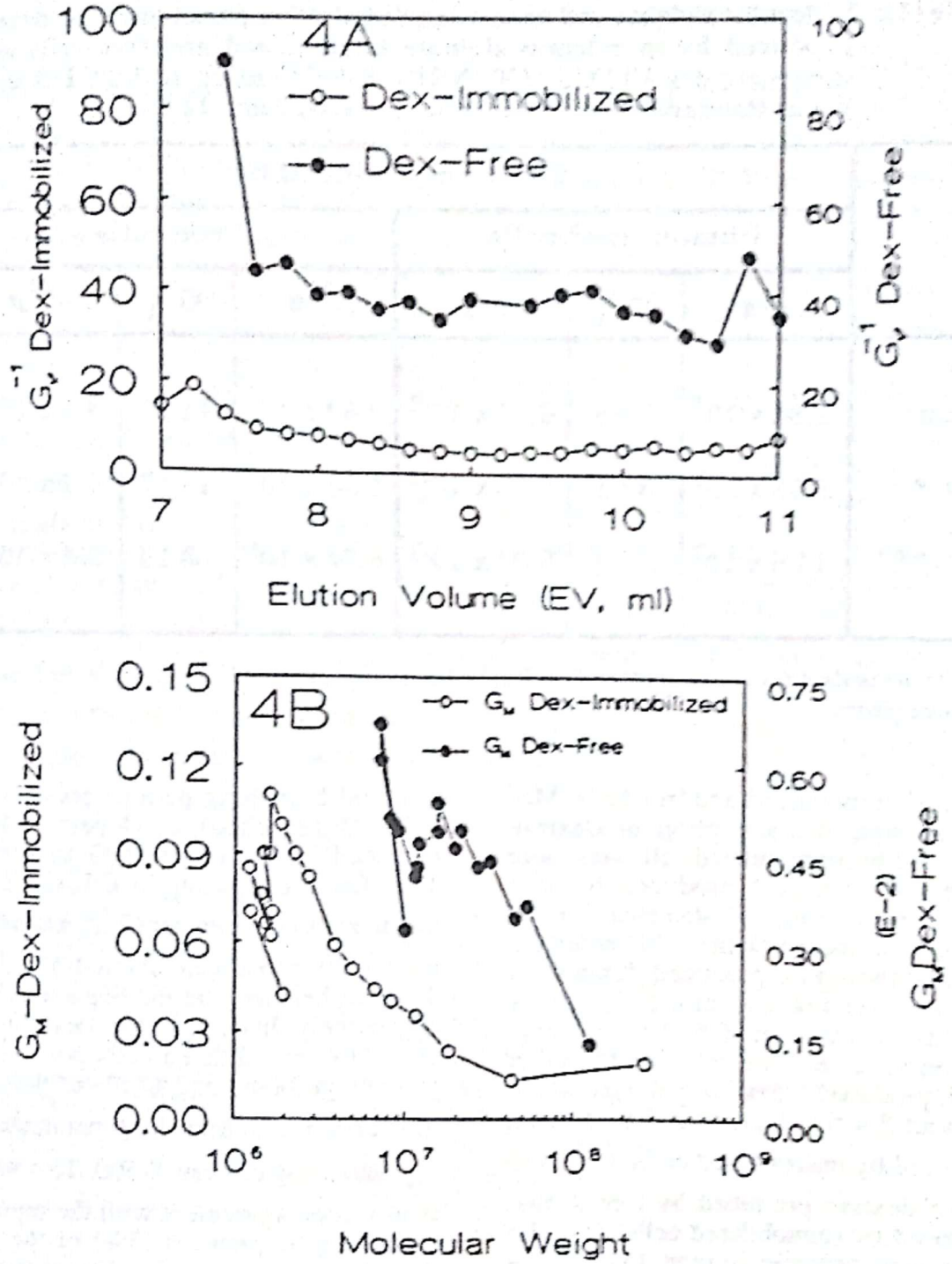

Figure (4): Plot of the branching parameters [G^{-1}_V vs. elution voluem, (A) and G_M vs. molecular weight; semilog plot (B)] for dextran produced by in calcium alginate immobilized and free cells of L. mesenteroides ATCC 10830 (NRRL B-512F) using dextran T-500 as a linear standard at elution volumes 7-11 ml.

Table (1): Molecular weights and branching distribution parameters for dextran produced by in calcium alginate immobilized and free cells of L. mesenteroides ATTC 10830 (NRRL B-512F) using dextran T-500 as a linear standard at elution volumes between 7 and 11 ml.

	Dextran produced by					
	Immobilized cells			Free cells		
	M_W	G^{-1}_V	G_M	M_W	G^{-1}_V	G_M
Mean	1.84×10^7	7.68	6.27×10^{-2}	2.43×10^7	41.21	4.5×10^{-3}
S.D.(*)	5.78×10^7	3.65	2.76×10^{-2}	2.86×10^7	13.17	1.2×10^{-3}
S.E.(**)	1.26×10^7	0.80	6.02×10^{-3}	6.74×10^6	3.10	2.8×10^{-4}

(*) S.D. = Standard deviation and (**) S.E. = Standard error were calculated using Slide Write Plus soft ware program.

broth of immobilized and free cells. Molecular weight distribution of dextran produced by immobilized cells was more dispersed than that produced by free cells. Considering the standard dextran T-500 as a linear polymer, the represented data show that produced dextran by immobilized cells is about 7.7 times heavier than dextran T-500 at the same elution volume. Free cells, on the other hand, produced a dextran polymer which is about 5.4 times heavier than dextran produced by immobilized cells (G^{-1}_V values of dextran produced by free to that produced by immobilized cells) with the same hydrodynamic volume. In comparison of dextran T-500 with that produced by free cells, a given volume of the latter is about 41.2 times heavier than similar volume of the former linear polymer. As reported by Yu and Rollings (1987)[9], when the degree of branching increases at a common elution volume, molecular weight increases as detected by LALLS due to an increase in the scattering centers in the molecule. Yu and Rollings compared the molecular weight distribution and branching parameters of amylose (0% branched), amylopectin (4-5% branched) and glycogen (10% branched). They found that, using SEC/LALLS and the branching parameter G^{-1}_V, amylopectin and glycogen were about 4-5 and 15-20 times heavier than the linear amylose, respectively. In comparison, dextran produced by immobilized cells possesses a percentage branching of about 6-9% as indicated by the branching parameter G^{-1}_V and using dextran T-500. This value is in a good agreement with the reported branching frequencies (5%) of the produced dextran by L. mesenteroides ATCC 10830 (NRR B-512F)[1-3]. Free cells on the other hand produced dextran which is more than 20% branched as detected by LALLS cells at elution volume between 7-11 ml.

Qualitative degree of branching of dextran produced by immobilized or free cells of L. mesenteroides ATCC 10830 (NRRL B-512F) was also performed through calculation of the branching par-

ameter G_M (the ratio of the mean-square radii of gyration between the linear and branched polymers). As reported by Yu and Rollings (1987)[9], amylopectin with 4-5% branches and glycogen with 10% branches possess G_M values of 0.05 and 0.01 compared with the linear amylose, respectively. Our results show that mean G_M value of dextran produced by immobilized cells is 0.067 ± 0.006 which can indicate a branching frequency with about 7% as above proposed by calculation of $G^{-1}{}_V$. For dextran formed by free cells, the mean G_M value is about 0.0045 which is inversely proportional with the branching frequency. Therefore, calculation of G_M for dextran produced by immobilized and free cells also supports a significant higher branching frequencies in dextran produced by free than immobilized cells.

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توزيع الوزن الجزيئي ومعدل التفريع في الدكستران المنتج بالخلايا الحره والخلايا المثبطه في اللجينات الكالسيوم من الليكرنوستك ميزنترويدس

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تم انتاج الدكستران بإستخدام الخلايا المثبطه في ٣٪ اللجينات الكالسيوم المكوره والخلايا الحره من ليكونوستك ميزنترويدس (ATCC 10830, NRRL B-512F) ، كما تم معرفة معلومات عن التركيب الجزيئي للمبلمر المكون بإستخدام كروماتوجرافيا التخلخل الحجمي المتصل علي التوالي بكاشف لأشعه الليزر ذات الزوايا المنخفضة. وقد وجد أن الدكستران المتكون خارج اللجينات الكالسيوم المكوره بواسطه الخلايا المثبطه له وزن جزيئي في المتوسط يبلغ ١٫٤٨ × 10^{7} دالتون. أما متوسط الوزن الجزيئي للدكستران المتكون بواسطه الخلايا الحره يبلغ مقداره ١٫٣٢ مره مثل ذلك المتكون بواسطه الخلايا المثبطه حيث بلغ مقداره ٢٫٤٣ × 10^{7} دالتوان. وبتقدير المعدلات النوعية للتفرع بإستخدام معاملي التفريع G^{-1}_{V} ، G_{M} وجد أن معدل التفرع في الدكستران المنتج بالخلايا الحره أعلي من معدل التفرع للدكستران المنتج بالخلايا المثبطه.