APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1979, p. 414-420
0099-2240/79/03-0414/07$02.00/0

Vol. 37, No. 3

Emulsifier of *Arthrobacter* RAG-1: Chemical and Physical Properties

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Received for publication 29 December 1978

The extracellular emulsifier of *Arthrobacter* RAG-1 was deproteinized by hot phenol treatment and purified by fractional precipitation with $(NH_4)_2SO_4$. The active fraction, precipitating between 30 and 35% saturation [EF-RAG(UET) WA], appeared to be homogeneous by immunodiffusion and sedimentation analysis. EF-RAG(UET)WA had an intrinsic viscosity of 750 cm^3/g, a sedimentation constant of 6.06S, a diffusion constant of 5.25×10^{-8} cm^2 s^{-1}, and a partial molar volume of 0.712 cm^3 g^{-1}. From these data a weight average molecular weight of 9.76×10^5 and a viscosity average molecular weight of 9.88×10^5 were calculated. EF-RAG(UET)WA contained 46.7% C, 7.01% H, and 6.06% N. Titration of the nonreducing polymer gave a single inflection point (pK' = 3.05), corresponding to 1.5 μmol of carboxyl groups per mg. Direct estimation of O-ester and hexose content of the highly acidic polymer yielded 0.65 and 0.29 μmol/mg, respectively. Mild alkaline hydrolysis released fatty acids with an average molecular weight of about 231. Strong acid hydrolysis of EF-RAG(UET)WA yielded D-glucose (minor), D-galactosamine (major), and an unidentified amino uronic acid (major).

Growth of *Arthrobacter* sp. RAG-1, like many other hydrocarbon-degrading microorganisms, is accompanied by emulsification of hydrocarbon in the growth medium (4, 12, 27). The emulsification was due to the production of an extracellular non-dialyzable material which was prepared by heptane extraction or precipitation between 30 and 40% $(NH_4)_2SO_4$ saturation (15). For the partially purified emulsifier to induce hydrocarbon in water emulsions efficiently, the hydrocarbon substrate must contain both aliphatic and cyclic components (14). A better understanding of the structure-function relationships governing this hydrocarbon substrate specificity requires a detailed characterization of the bioemulsifier itself. In the studies presented here, the emulsifier has been purified to apparent homogeneity and certain of its chemical, physical, and immunological properties have been measured.

MATERIALS AND METHODS

Determination of emulsification activity. Emulsion formation was measured as previously described (15) in 125-ml flasks containing 25 to 400 μg of emulsifying agent, 0.1 ml of 1:1 (vol/vol) hexadecane and 2-methylnaphthalene, and 0.05 M tris(hydroxymethyl)aminomethane (Tris) buffer (pH 7.4), containing 10 mM $MgSO_4$ to a final volume of 7.5 ml. Flasks were agitated by reciprocal shaking (150 strokes per min) for 1 h at 26°C. Contents of the flasks were then transferred to Klett tubes for measurement of turbidity in a Klett-Summerson colorimeter fitted with a green filter. A unit of activity according to this assay yields 13.3 Klett units; specific activity is given in units per milligram (dry weight).

General analytical methods. Protein concentrations were determined by the method of Lowry et al. (8), using bovine serum albumin as a standard. Hexoses were determined by the indole-sulfuric acid method (2), using D-glucose as the standard. Hexosamines and reducing sugars were estimated after hydrolysis in 5 N HCl for 30 min at 100°C; total hexosamines was determined by the Partridge modification (24) of the Elson-Morgan method, using galactosamine as the standard; and total reducing sugars were estimated by the Somogyi-Nelson arsenomolybdate method (19), using galactosamine as the standard. *O*-acyl was estimated by a modification (20) of the Hestrin method (6), using acetohydroxymate as the standard. Periodate oxidation was followed spectrophotometrically (9) in a 2.0-ml solution (0.1 M acetate buffer, pH 4.5).

A Beckman model E analytical ultracentrifuge equipped with a schlieren optical system was used for measurement of sedimentation velocity and diffusion constant at 19 ± 0.2°C. Absorbance was read on a Gilford model 240 spectrophotometer. Density measurements were performed with the Digital Densimeter DMA002C (1).

Viscosity was measured on 1.0-ml samples in an Ostwald-Fenske microviscometer (water value, 47.8 s) at 26.4°C. Titrations were carried out with a pH meter by a microprocedure in which 0.01-ml portions of 0.1 N HCl or NaOH solutions were added with thorough

mixing and exclusion of carbon dioxide to 4.0-ml solutions to be titrated.

Paper chromatography (Whatman no. 1 paper, descending technique, room temperature) was performed in two solvent systems: (A) *n*-butanol-pyridine-water (6:4:3, vol/vol); (B) *n*-propanol-ethyl acetate-water (7:1:2, vol/vol). Multiple chromatograms were searched for (i) sugars and polyols by alkaline silver nitrate (3), (ii) amino acids and hexosamines by dipping in 0.2% solution of ninhydrin in acetone, which contains 2% pyridine, followed by heating at 105°C for 2 to 3 min (10), and (iii) amino sugars and their *N*-acetyl derivatives by spraying with alkaline acetylacetone followed by *p*-dimethylaminobenzaldehyde hydrochloride reagent (24).

D-Glucose and D-galactose derivatives were determined by spraying freshly prepared Glucostat and Galactostat reagents (products of Worthington Biochemicals Corp., Freehold, N.J.) on the dried chromatogram.

Immunological methods. For the preparation of antibody, rabbits were injected with 1 mg of EF-RAG(HD) in 1 ml of complete Freund adjuvant. After 2 weeks the rabbits were injected again with the same quantity of EF-RAG(HD) in complete Freund adjuvant. The rabbits were bled 11 to 14 days later. A crude immunoglobulin fraction was obtained by $(NH_4)_2SO_4$ fractionation. Control sera were obtained from unimmunized rabbits. Immunodiffusion was performed on 76- by 26-mm microscope slides, using 1% agar in distilled water.

Materials. Preparation of extracellular emulsifying agents of *Arthrobacter* sp. RAG-1, referred to as EF-RAG(UET) and EF-RAG(HD), were described previously (15). EF-RAG(UET) and EF-RAG(HD) refer to materials prepared from cultures growing on ethanol and hexadecane, respectively. Bovine serum albumin, alcohol dehydrogenase, hexokinase, and lysozyme were purchased from Worthington Biochemicals Corp. Sephadex G-100 was from Pharmacia Fine Chemicals Inc. Other commercially available chemicals were of reagent-grade quality.

RESULTS

Preparation of the deproteinized emulsifying agent EF-RAG-W. Various samples of the extracellular emulsifying agent of RAG-1, referred to as EF-RAG(UET), contained 5 to 15% protein by weight. To ascertain whether or not the protein moiety was essential for emulsifying activity, protein was separated from the bulk of material by a modification of the hot phenol method (23). One gram of EF-RAG(UET) dissolved in 200 ml of water with the aid of a few drops of concentrated NH_4OH was brought to 65 to 68°C and added to an equal volume of 90% phenol (preheated to 65°C). The mixture was stirred vigorously for 15 min at 65°C and then cooled to 10°C in an ice bath. The resulting emulsion was centrifuged at 5,000 × *g* for 30 min. After transferring the viscous aqueous phase to a flask, the remaining phenol layer and interface were extracted three more times with 200 ml of water. The combined water extracts were dialyzed extensively against several changes of distilled water and then freeze-dried to obtain a white fluffy material referred to as EF-RAG(UET)-W (yield, 850 mg [85%]). The remaining phenol fraction and interphase were suspended in water, dialyzed extensively against distilled water, and freeze-dried. The resulting yellowish proteinaceous material was referred to as EF-RAG(UET)-P (yield, 100 mg [10%]).

After hot phenol treatment, all of the recoverable emulsifying activity (45%) was found in the aqueous phase, i.e., EF-RAG(UET)-W (Table 1). None of the activity was associated with the denatured protein fraction. However, addition of 0.2 and 2.0 μg of EF-RAG(UET)-P per ml to 10 μg of EF-RAG(UET)-W per ml resulted in 25 and 66% stimulations in emulsifying activity, respectively. The stimulation by EF-RAG-(UET)-P was not specific since several different proteins, such as bovine serum albumin, lysozyme, hexokinase, and denatured alcohol dehydrogenase, also stimulated EF-RAG(UET)-W emulsification of gas oil.

Ammonium sulfate fractionation of deproteinized EF-RAG(UET). The phenol method described above was repeated on 820 mg of EF-RAG(UET). After three phenol extractions, the combined water extracts were extracted four times with an equal volume of ether to remove residual phenol. After evaporation of residual ether from the aqueous phase by bubbling with nitrogen, the viscous solution was cooled to 5°C and brought to 32.5% ammonium sulfate saturation. (No precipitate formed at 30% saturation.) After standing for 1 h at 5°C, the clear translucent precipitate was collected by centrifugation at 5,000 × *g* for 30 min at 5°C. The procedure was repeated to obtain a slightly turbid second precipitate between 32.5 and 35% saturation and another small amount of precipitate between 35 and 40% saturation. No addi-

TABLE 1. *Emulsification of gas oil by deproteinized EF-RAG(UET)*

Emulsifier[a]	Sp act[b] (U/mg)
EF-RAG(UET)	276
EF-RAG(UET)-P	0
EF-RAG(UET)-W	146

[a] Experiments were performed using 75 μg of EF-RAG(UET), 75 μg of deproteinized emulsifier, EF-RAG(UET)W, and 15 μg of denatured protein obtained by phenol extraction, EF-RAG(UET)P.

[b] Emulsification of gas oil was measured in 7.5 ml of 0.2 M Tris buffer (pH 7.4) containing 10 mM $MgSO_4$.

tional precipitate formed between 40 and 60% saturation. Each of the precipitates was dissolved in water. They and the remaining 60% saturated solution were dialyzed at 2 to 5°C successively against distilled water, 0.05 N HCl (24 h), and double-distilled water. The resulting solutions were freeze-dried and analyzed (Table 2).

Over 99% of the recovered emulsifying activity of EF-RAG(UET)-W precipitated in the two fractions between 30 and 35% ammonium sulfate saturation. These two fractions contained similar specific activities of emulsifier and exhibited the same proportions of O-ester, carboxylic acid, and hexose. None of the fractions contained significant quantities of protein. The active fractions had high specific viscosities. The fractions precipitating between 30 and 35% saturation constituted 85% recovery of the input activity.

Immunological properties of EF-RAG. The homogeneity of the different preparations of EF-RAG was examined by immunodiffusion. Antibodies prepared against EF-RAG(HD) cross-react with the deproteinized and ammonium sulfate-fractionated EF-RAG(UET). The two active fractions, precipitating between 30 to 32.5% and 32.5 to 35% ammonium sulfate saturation, each gave a single identical band upon Ouchterlony two-dimensional diffusion. The minor fractions that precipitated between 35 and 40% saturation and contained poor emulsifying activity gave a band that diffused more rapidly than the 30 to 35% fractions. Since the two fractions precipitating between 30 and 35% saturation were similar chemically (Table 2) and immunologically and had the same specific activity, they were combined and referred to as EF-RAG(UET)WA.

Chemical composition of EF-RAG. Elementary analyses of EF-RAG(UET) and EF-RAG(UET)WA are shown in Table 3. The latter sample released 12.7% water on drying at 55°C in vacuo. The deproteinized sample contained significantly less N, S, and ash than EF-RAG(UET). The C-N-H ratio of EF-RAG(UET)WA was calculated to be 9.0:1.0:16.1. No significant quantities (<0.5%) of phosphorous or halides were found in either sample. Functional group tests were positive for carboxyl and ester groups and negative for methoxy, ethoxy, and unsaturated groups. The polymer contained less than 0.02 μmol of reducing sugar per mg. The nonreducing polymer was resistant to high temperatures in neutral and alkaline conditions. No emulsifying activity was lost at 100°C for 2 h in distilled water; 50% of the activity remained even after treatment in 1 N NaOH at 100°C for 1 h. EF-RAG(UET)WA was considerably more sensitive to acid, losing 50% of its emulsifying activity in 2 min at 100°C in 1 N HCl.

Titration of EF-RAG(UET)WA (40 mg/4 ml) between pH 2.5 and 10.5 showed a single inflection point, corresponding to pK′ = 3.05 (identical to a standard sample of glucuronic acid).

EF-RAG(UET)WA consumed 0.24 μmol of periodate per mg. Periodate uptake ceased after 2 h at 30°C, pH 4.5. The periodate-treated material did not lose any emulsifying activity.

Physical characterization of EF-RAG-(UET)WA. Preliminary experiments indicated that the purified emulsifying agent was excluded by Sephadex G-100 and G-200 and did not pass an Amicon XM-30 filter. This plus the fact that the deproteinized non-dialyzable emulsifying agent contained 1.5 μmol of carboxylic groups per mg suggested that it was an anionic polymer. The intrinsic viscosities of EF-RAG(UET), EF-RAG(UET)W, and EF-RAG(UET)WA in 0.15

TABLE 3. *Elementary composition of EF-RAG*

Sample[a]	Composition				
	% C	% H	% N	% S	% Ash
EF-RAG(UET)	41.72	6.95	7.74	0.7	13.8
EF-RAG(UET)WA	46.70	7.01	6.06	0.0	3.5

[a] Analyses were performed on samples that had been dried to constant weight at 55°C in vacuo. The results are the average of at least three determinations.

TABLE 2. *Analysis of ammonium sulfate fractions of deproteinized EF-RAG(UET)*[a]

$(NH_4)_2SO_4$ fraction (% saturation)	Wt (mg)	Emulsifying activity		Reduced viscosity (cm³/g)	Protein (%)	O-ester (μmol/mg)	Carboxylic acid (μmol/mg)	Hexose (μmol/mg)
		U	Sp act					
30–32.5	379	66,500	175	810	0.3	0.66	1.5	0.27
32.5–35	194	34,500	178	570	0.15	0.63	1.5	0.33
35–40	25	780	31	400	0.5	0.81		0.20
40-sol.[b]	82	0	0		0.7			0.08

[a] A 820-mg amount of EF-RAG(UET) (specific activity, 146 U/mg) was deproteinized by the hot-phenol method, fractionated by addition of solid $(NH_4)_2SO_4$, converted to the acid form by dialysis against 0.05 N HCl and water, freeze-dried, and analyzed as described in the text.

[b] 40-sol. is that fraction which did not precipitate at 40% $(NH_4)_2SO_4$ saturation.

M Tris buffer, pH 7.4, were 470, 505, and 750 cm^3/g, respectively. With all three samples, specific viscosity was independent of concentration between 0.05 and 1.0 mg/ml. Exposure of 0.5 mg of EF-RAG(UET)W per ml to sonic oscillations (Branson B12 Sonifier, setting 8, 14 min) reduced the viscosity to 420 cm^3/g. Exposure for an additional 20 min did not further reduce the viscosity. The viscosity of EF-RAG-W as a function of ionic strength is shown in Fig. 1. Between 0.03 and 0.15 M NaCl, viscosity decreased slightly from 515 to 480 cm^3/g. The large increase in specific viscosity at low ionic strengths is characteristic of polyelectrolytes and has been attributed to dilution of counterions (21). Specific viscosity was also measured as a function of pH, using 0.05 M citrate-phosphate buffer (pH 3 to 7) and 0.05 M Tris-hydrochloride buffer (pH 6.8 to 8.5). Throughout the entire range (pH 3 to 8.5) the viscosity of EF-RAG(UET) remained at 480 ± 50 cm^3/g.

Sedimentation velocity analysis of 2 mg of EF-RAG(UET)WA per ml in 0.15 M NaCl showed a single broad band corresponding to an s_{20} of 6.06×10^{-13} s or 6.06S. The diffusion coefficient, D, also determined in the analytical centrifuge, was 5.25×10^{-8} cm^2 s^{-1}. The partial specific volume of the material, $\bar{V}$, was 0.712 cm^3 g^{-1}.

Estimating the molecular weight of EF-RAG(UET)WA from the equation, $M = RTs/D(1 - \bar{V}\rho)$, where R is the gas constant, T is the absolute temperature, and ρ is the density of the solution, yields a weight average molecular weight of 9.76×10^5. Alternatively, the molecular weight can be estimated using the determined values for intrinsic viscosity, η, sedimentation constant, S, and partial specific volume, $\bar{V}$, according to the equation of Scheraga and Mandelkern (16): $M^{2/3} = Ns\, \eta_i^{1/3}\eta_s/100^{1/3}\beta'(1 - \bar{V}\rho)$, where N is Avogadro's number, η_s = viscosity of the solvent, and $\beta' = 2.5 \times 10^6$. The calculated viscosity average molecular weight for EF-RAG-(UET)WA was 9.88×10^5.

The ultraviolet absorption spectrum of EF-RAG(UET)WA (220 to 350 nm) showed no maxima. The infrared spectrum of EF-RAG(UET)W incorporated into a KBr pellet or nugol revealed the following groups: 3,340 cm^{-1} (O—H), 2,900 cm^{-1} (C—H), 1,720 cm^{-1}, weak (C═O), 1,640 cm^{-1} (amide I), and 1,545 cm^{-1} (amide II).

Alkaline hydrolysis of EF-RAG-(UET)WA. Two hundred milligrams of EF-RAG(UET)WA was refluxed in 40 ml of 1 N NaOH for 4 h, cooled, extracted three times with 40 ml of diethyl ether, acidified to pH 1 to 2 with concentrated HCl, and extracted again three times with 40 ml of ether. The acid-ether extracts were combined and dried in a tared flask,

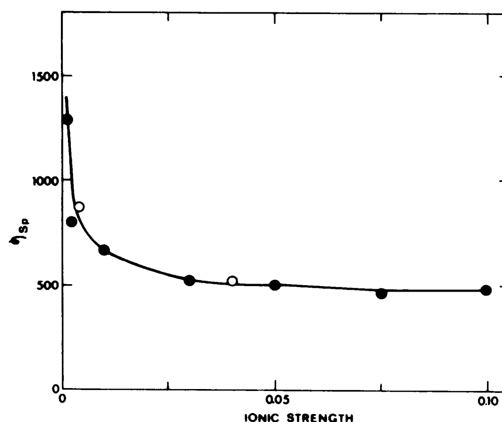

FIG. 1. *Reduced viscosity of EF-RAG-W as a function of ionic strength. Solutions of EF-RAG(UET)WA (0.5 mg/ml) were prepared in distilled water containing varying concentrations of NaCl (●) or $MgSO_4$ (○). Reduced viscosities, η_{sp}, were determined as described in the text. Ionic strength is ½ $\sum m_i z_i^2$, where m_i is the molality and z_i is the charge of each ionic species.*

yielding 30 mg (15%) of fatty acids; extraction with ether before acidification yielded less than 2 mg of dry material. Combining the weight recovery of fatty acid from the polymer (150 μg/mg) and the O-ester content (0.65 μmol/mg) yields an average equivalent weight of 231 for the fatty acid(s).

Acid hydrolysis of EF-RAG(UET)WA. Preliminary hydrolysis studies were performed at 80 and 100°C in sealed tubes with concentrations of hydrochloric acid varying from 0.01 to 6.0 M. After removal of HCl in vacuo, the products were examined for reducing power and amino sugars and by paper chromatography in solvents A and B. At 0.05 M HCl and 100°C (Fig. 2), there was a release of around 6% reducing sugar during the first hour, followed by a slower release of about 1% reducing sugar per h for the next 20 h. After 27 h of hydrolysis in 0.05 M HCl at 100°C, chromatography revealed the presence of two major reducing spots (subsequently identified as galactosamine and an aminouronic acid) and one minor component (subsequently identified as glucose). In addition, there were considerable amounts of incompletely hydrolyzed material (remaining near the origin). After 5 h of hydrolysis in 0.05 M HCl, only glucose was detected on the chromatograms. *N*-acetylated derivatives of the amino sugars were never detected. No attempt was made to detect fatty acids on the chromatograms.

A maximum amount of reducing sugar was obtained by hydrolyzing EF-RAG(UET)W in 5

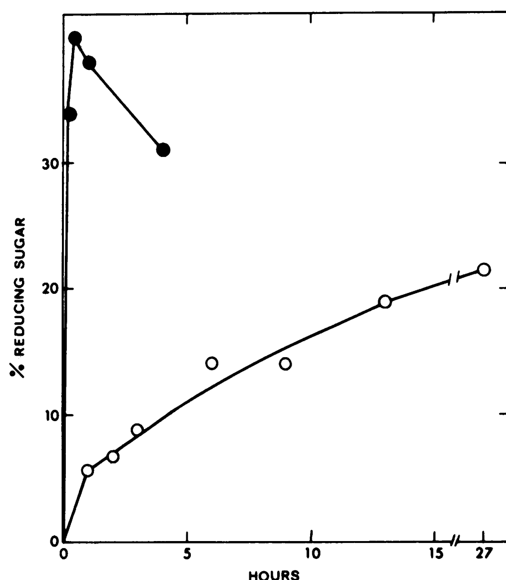

FIG. 2. *Acid hydrolysis of EF-RAG(UET)W. The weight percentage of reducing power is plotted against the duration of hydrolysis at 100°C at 0.05 N HCl (○) and 5 N HCl (●). Hydrolyses were performed in sealed tubes under nitrogen on 1-mg/ml samples of the emulsifier.*

M HCl at 100°C for 30 min. Even under these conditions significant amounts of emulsifying agent were incompletely hydrolyzed. This was demonstrated by eluting material from the origin of the chromatograph and hydrolyzing it a second time. Additional amino sugars were released from the incompletely hydrolyzed sample. Longer periods of hydrolysis resulted in further destruction of the sugars. The relative amount of amino sugars to glucose increased with time of hydrolysis due to both the slower release of amino sugars from the polymer and faster destruction of free glucose. Hydrolysis of samples obtained by ammonium sulfate fractionation (Table 2) showed the same chromatographic pattern as EF-RAG(UET)W. After hydrolysis in 5 M HCl at 100°C for 30 min, EF-RAG(UET)WA released 37.6% reducing sugar and 24.4% total hexosamines (in both cases, galactosamine was used as the standard).

Identification of sugar components of EF-RAG(UET)W. Table 4 summarizes the data that led to the conclusion that the sugars produced by hydrolysis of EF-RAG(UET)W were D-glucose (minor), D-galactosamine (major), and an amino uronic acid (major). Unknown compound i did not separate from glucose in solvents A or B and yielded a positive D-glucose reaction directly on the paper. Unknown compound ii migrated identically to galactosamine in solvent B, gave a positive D-galactose oxidase reaction (17), and was converted to lyxose (R_{Glc} = 1.49 in solvent B) by ninhydrin degradation (24). Unknown compound iii gave positive reactions for reducing sugar, amino sugar, and carboxylate ion. Moreover, it was similar both in chromatographic behavior and in its reaction with the nitrous acid-indole test (2) to 2-amino-2-deoxyhexuronic acids (13, 25).

DISCUSSION

The extracellular emulsifying factor of *Arthrobacter* sp. RAG-1 was deproteinized by hot phenol treatment and purified by fractional precipitation between 30 and 35% ammonium sulfate saturation. The resulting acidic polysaccharide preparation was free of protein and nucleic acids and appeared to be homogeneous by several criteria. (i) Only a single band was found by Ouchterlony two-dimensional diffusion. (ii) Only a single component was observed by sedimentation velocity studies, using several concentrations of material. (iii) Attempts to further purify the material by extraction or precipitation with organic solvent did not improve its specific activity or alter its chemical composition.

The *Arthrobacter* RAG-1 emulsifier is a highly acidic polysaccharide with a molecular-weight average close to 10^6. Molecular-weight determination from sedimentation and diffusion data closely fit the value obtained from a consideration of sedimentation and viscosity measurements. In both cases the determined value for the partial specific volume of 0.712 cm^3 g^{-1} was used. The relatively high intrinsic viscosity, low

TABLE 4. *Properties of sugar products of EF-RAG(UET)W hydrolysis*[a]

Component[b]	R_{Glc}[c]	Reaction
Standards		
D-Glucose	1.25	Glucose oxidase
D-Galactose	1.22	Galactose oxidase
D-Glucosamine	1.00	Ninhydrin (purple), Elson-Morgan, glucose oxidase
D-Galactosamine	0.85	Ninhydrin (purple), Elson-Morgan, galactose oxidase
D-*N*-acetylgalactosamine	1.58	Elson-Morgan
Acid hydrolysis products of EF-RAG (UET)W		
i	1.25	Glucose oxidase
ii	0.85	Ninhydrin (purple), Elson-Morgan, galactose oxidase
iii	0.23	Ninhydrin (greenish-yellow, later blue), Elson-Morgan

[a] Obtained after 24 h of hydrolysis of EF-RAG(UET)W in 0.05 M HCl at 100°C.
[b] All components gave positive alkaline silver nitrate tests. Spot tests were determined directly on the chromatograms.
[c] Rate of movement of each sugar relative to glucosamine in solvent A.

diffusion constant, and low sedimentation coefficient of the emulsifier indicate that the shape of the polymer is highly asymmetrical. Using Simha's factor (21) for the viscosity increment of rod-shaped ellipsoids indicates that the polymer has an axial ratio of close to 100. Preliminary examination of the purified emulsifier by electron microscopy revealed thin fibers with lengths greater than 100 nm.

The polymer is composed of two major sugars (D-galactosamine and an unidentified amino uronic acid), one minor sugar (D-glucose), and an unidentified fatty acid ester (Table 5). Titration curves and the infrared spectrum of the polymer indicate that the amino sugars are N-esterified. The amino uronic acid content of the polymer was estimated by acid-base titration of the polymer to be 1.5 μmol/mg. Assuming the compound to be an *N*-acetylhexosamine uronic acid (molecular weight of 222), it would comprise 33% (by weight) of the polymer. Direct estimation of D-galactosamine content of the polymer is not possible at this time since hydrolysis conditions necessary to release it from the polymer cause considerable decomposition of the amino sugar. Resistance to hydrolysis of polymers containing amino sugars and uronic acids is well known (18). Rough estimates (from intensities of reducing and ninhydrin-positive materials on chromatograms) indicate that the amount of D-galactosamine is similar to the quantity of amino uronic acid. The D-glucose content of the polymer was 0.29 μmol/mg or 5.2% (by weight). The strong acid conditions (hot sulfuric acid) used to estimate hexose content should release all of the glucose from the polymer. The fatty acid ester content of the polymer was 15% (by weight), with an average equivalent weight of about 231.

The chemical composition of EF-RAG-(UET)WA differs from the few previously described microbiologically produced surfactants and emulsifiers (4, 27). Although hexosamine uronic acids are not common constituents of polysaccharides, they have been reported previously in the type-specific substance of *Haemophilus influenzae* type d (26), lipopolysaccharides from *Pseudomonas aeruginosa* (25), K antigens of *Vibrio parahaemolyticus* (22), surface polysaccharide antigens of *Staphylococcus aureus* M (5, 7), and the cell walls of *Micrococcus lysodeikticus* (11) and *Halococcus* sp. strain 24 (13).

TABLE 5. *Chemical composition of EF-RAG(UET)WA*

Component	% of polymer
D-Galactosamine[a]	(20–30)[b]
Amino uronic acid[a]	33.3
D-Glucose	5.2
Fatty acid ester	15.0
Water	12.7
Ash	3.5

[a] Calculated as *N*-acetylated amino sugar.

[b] Estimated from intensity of ninhydrin and reducing spots on chromatograms.

ACKNOWLEDGMENTS

We thank M. M. David and E. Daniel, Biochemistry Department, Tel Aviv University, for performing and assisting in the interpretation of sedimentation and partial molar volume measurements, I. Witz and his group for help with the immunological studies, the Weizmann Institute of Science for performing the elementary analyses, and R. Avigad for excellent technical assistance.

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