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CHARACTERIZATION OF GUM ARABIC FRACTIONS OBTAINED BY ANION-EXCHANGE CHROMATOGRAPHY

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Key Word Index—*Acacia senegal*; Leguminosae; gum arabic; anion-exchange chromatography; DEAE-cellulose; arabinogalactan proteins.

Abstract—Two samples of authentic gum arabic (A and B) have been fractionated by anion-exchange chromatography on DEAE-cellulose. The fractions were isolated by a step-wise increase in the ionic strength of the elution buffer. Samples A and B yielded five and six fractions, respectively. All of the fractions isolated were polydisperse containing varying proportions of the different M_r species associated with the whole gum. The carbohydrate composition of all fractions remained relatively constant with each containing similar proportions of galactose, arabinose, rhamnose and glucuronic acid. The protein content of the fractions varied slightly (0.31–2.8%). For sample A, the protein content decreased in the order F2 > F1 > F3 > F5 > F4; for sample B the order was F1 > F6 > F3 > F2 > F4 > F5. Whereas Hyp and Ser were the principal amino acids for the whole gums and the majority of the fractions isolated, two of the fractions from sample A had Asp, Ser and Glu as their principal components. Interaction of the fractions from both samples with an artificial carbohydrate antigen (Yariv reagent) indicated that they all contained arabinogalactan proteins (AGP). In addition, immuno-dot blots of these fractions screened against a panel of anti-AGP monoclonal antibodies demonstrated that they all contained epitopes recognized by one or more of these antibodies. These data describe the results of the first chemical characterization of the molecular components of gum arabic fractionated using anion-exchange chromatography. They further demonstrate the molecular complexity of this widely studied natural product, and in combination with other separation procedures may provide the key to the eventual elucidation of the biochemical synthesis of this fascinating and useful substance.

INTRODUCTION

Despite the wealth of information which has been reported describing the overall chemical structure of the gum exudate from *Acacia senegal* (gum arabic) [1, 2] little is currently known of the individual components which together constitute the gum *in toto*, and still less about its mechanism of biosynthesis ('gummosis') and the biological significance of this process.

Chemical studies of the gum have demonstrated that it consists mainly of a highly complex polysaccharide of a branched β-(1, 3)-linked galactose backbone with branches linked through the 1,6-positions, with arabinose, rhamnose and uronic acids in ramified side-chains [3]. This 'complex' also contains small amounts of protein which include arabinogalactan-proteins (AGPs) [4, 5]. Recent studies of gum arabic fractionated using hydrophobic affinity chromatography have divided the gum into three fractions. These fractions were referred to as an AGP-rich fraction (10.4% of total gum), an arabinogalactan fraction (88.4% of total gum) and a glycoprotein fraction [6, 7].

Connolly and co-workers [8, 9] presented results from enzymatic degradation studies of the gum which were in agreement with the 'wattle blossom' model for AGP structure originally proposed by Fincher *et al.* [4], in which large arabinogalactan side-chains are attached to a common core protein. However, more recently Qi *et al.* [10] reported the isolation of a high M_r glycoprotein from the gum, which, whilst containing polysaccharide side-chains characteristic of AGPs, had an amino acid composition more closely resembling extensin, being low in Ala but rich in Ser and His. This interesting glycoprotein was shown to have a 'hairy rope' structure. It is possible that both these structures exist within the gum and other recent studies have demonstrated the presence of a multitude of yet to be identified proteins in various gum samples [11], which suggests that the structure of the gum may be far more complex than previously proposed.

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Earlier studies concerned with gum biosynthesis suggested that the gum was formed from starch [12]; however, this was later refuted by Anderson and Dea [13] who pointed out that the enzymatic pathways necessary for such a transformation are too complex to be feasible. They proposed that the bulk of the gum had as its precursor a highly branched arabinogalactan to which is added rhamnose, uronic acids and 4-*O*-methylglucuronic acid-terminated side-chains. Anatomical studies of gum formation have been reported by Ghosh and Purkayastha [14] and more recently by Joseleau and Ullmann [15, 16]. Ghosh and Purkayastha [14] showed that the gum forms in cysts in the inner bark and that the development of these cysts is preceded by changes in the adjacent parenchymatous tissue. The actual site of gum synthesis is proposed to be the inner phloem. Joseleau and Ullmann [16] have extended these earlier studies to a chemical analysis of various tissues from 'gum-productive' and 'non-productive' tree branches. They demonstrated the presence of gum in cambial tissue and also showed that the gum was not restricted to the site of exudation, but was also present in tissues some distance from the scarification of the branch. However, the actual chemical/biochemical synthesis of the gum still remains to be elucidated. Before experiments can be designed to study this process further, we must first isolate and characterize all of the various molecular components of the gum. In order to facilitate such analyses, methods need to be developed in order to fractionate the gum into its 'component parts'.

With this aim in mind we present the results of further investigations of the molecular complexity of gum arabic fractionated by ion-exchange chromatography on DEAE-cellulose. This technique has been used previously [17] to demonstrate the heterogeneity of the gum but no attempt was made to characterize the fractions obtained. In the present study, the gum fractions have been extensively characterized using an array of physicochemical and immunochemical techniques; the results obtained are discussed in relation to current knowledge of the structure and composition of gum arabic.

RESULTS

Fractionation on DEAE-cellulose

For both samples, a significant proportion of the gum (*ca* 60%) did not bind to the column and passed through in the mobile phase. This was collected and is referred to as fraction 1. Further fractions were eluted by increasing the ionic strength of the elution buffer. For sample A, no further elution was observed when the ionic strength of the buffer exceeded 0.2 M NaCl, indicating that the gum had fully desorbed from the gel matrix. For sample B, a further fraction eluted with 0.29 M NaCl. The total yield for samples A and B was 84% and 75%, respectively, which is considered to be satisfactory in view of losses which can occur during the fractionation and dialysis procedures. The yields of each of the fractions are reported in Table 1 and varied between the two samples.

Chemical characterization

The specific rotation, composition of neutral sugars and glucuronic acid content of the gums and their fractions are given in Table 2. The analysis of the whole sample is consistent with the literature data from *A. senegal* gum obtained from Sudan [18]. Fractions of both samples show similar specific rotation values and contain similar proportions of neutral sugars which suggests that the carbohydrate structure is relatively constant in all fractions.

The glucuronic acid content of the whole gum samples was found to be *ca* 17%. Small variations in the glucuronic acid contents of the fractions of both samples were detected and interestingly fraction 2 of each sample had a significantly lower value compared to the others.

Table 1. Yield of gum arabic fractions prepared by ion-exchange chromatography

Fraction number	Elution buffer	Weight recovered (g)	% of total yield
Sample A			
Fraction 1	1.0 mM PB*	8.10	56.6
Fraction 2	0.08 M NaCl in PB	0.60	4.2
Fraction 3	0.12 M NaCl in PB	1.96	13.7
Fraction 4	0.16 M NaCl in PB	2.34	16.4
Fraction 5	0.20 M NaCl in PB	1.30	9.1
Total weight		14.30	84.3
Sample B			
Fraction 1	1.0 mM PB	7.64	60.5
Fraction 2	0.08 M NaCl in PB	4.12	32.6
Fraction 3	0.12 M NaCl in PB	0.23	1.9
Fraction 4	0.16 M NaCl in PB	0.33	2.6
Fraction 5	0.20 M NaCl in PB	0.27	2.2
Fraction 6	0.29 M NaCl in PB	0.043	0.34
Total weight		12.64	75.2

*PB = 1 mM phosphate buffer.

Table 2. Specific rotation and carbohydrate composition of gum arabic samples A and B and IEC fractions

Sample	$[\alpha]_D$	Rha (%)	Ara (%)	Gal (%)	GlcA (%)
Sample A	− 30.0	12 ± 2	24 ± 1	38 ± 3	17 ± 1
Fraction 1	− 28.0	16 ± 1	25 ± 2	32 ± 2	20 ± 1
Fraction 2	nd*	15 ± 1	27 ± 2	32 ± 1	15 ± 1
Fraction 3	− 29.0	13 ± 1	27 ± 1	40 ± 2	20 ± 2
Fraction 4	− 31.0	14 ± 1	25 ± 1	38 ± 2	19 ± 2
Fraction 5	− 32.0	14 ± 1	27 ± 2	32 ± 1	21 ± 1
Sample B	− 30.0	12 ± 1	25 ± 1	42 ± 2	17 ± 1
Fraction 1	− 32.0	12 ± 1	24 ± 1	42 ± 2	14 ± 2
Fraction 2	nd	13 ± 1	26 ± 1	36 ± 2	9 ± 2
Fraction 3	nd	14 ± 1	25 ± 1	38 ± 2	15 ± 2
Fraction 4	− 27.0	14 ± 1	23 ± 1	42 ± 2	15 ± 2
Fraction 5	− 28.0	14 ± 1	22 ± 1	40 ± 2	19 ± 1
Fraction 6	− 33.0	15 ± 1	23 ± 1	42 ± 1	19 ± 1

*nd = not determined.

Amino acid composition

Amino acid analysis of the whole gum samples (Tables 3 and 4) showed that Hyp and Ser were the major amino acids and accounted for *ca* 41% and 44%, respectively, of the amino acid complement of each gum. The total N content of samples A and B was 0.3% and 0.35%, respectively. These values fall within the range of 0.27% to 0.39% specified for gum arabic by JECFA [19] and are typical for Sudanese gums [18]. The N conversion factors (NCF) were calculated from the protein:nitrogen ratio and were 6.7 and 6.8 for samples A and B, respectively; these are similar to literature values [20].

For the fractions, the principal amino acids for sample A fractions 1, 4 and 5 and sample B fractions 1–6 were Hyp and Ser. In fact, the distribution in all of these fractions was similar to that previously reported by Qi *et al.* [10] in that they had a Hyp_4 Ser_2 Thr Pro Glu Leu His repeat unit. For sample A, however, fractions 2 and 3 had Asp, Ser, Glu, Gly and Leu as their principal components. The protein content of the fractions decreased in the order F2 > F1 > F3 > F5 > F4 for sample A, and in the order F1 > F6 > F3 > F2 > F4 > F5 for sample B.

M_r *distribution*

The M_r distributions of whole gum samples A and B as monitored by UV (A_{206} nm) and by RI are given in Figs 1a and b. The distribution is typical of that reported previously for *A. senegal* gum [21]. The RI signal closely reflects the concentration of the gum and, hence, gives a better indication of the 'true' M_r distribution. UV detection is more sensitive to the chemical components within the various M_r entities of the gum and in particular shows enhanced absorbance due to the proteinaceous components present. Two M_r peaks are detected by RI; the major peak (peak A) elutes at $K_{av} = 0.33$, the minor one (peak B) at $K_{av} = 0.04$.

Three M_r peaks are detected by UV. Peaks A and B correspond to the two peaks observed using RI detection. In addition, a third peak is present, (peak C) at $K_{av} = 0.46$. The UV intensities of peaks B and C are high compared with peak A due to the presence of protein, as reported previously. These peaks were more prominent in sample B which is indicative of its higher protein content (Tables 3 and 4).

The GPC chromatograms of the fractions of the two samples are shown in Figs 2a and b, respectively. It is interesting to note that all the fractions are polydisperse, each containing varying proportions of the three M_r species. For both samples, the protein-rich M_r species corresponding to peak B are collected mainly in fraction 1. However, fraction 1 of sample B also contains a significant proportion of the protein-rich peak C species (as noted from the UV profile). For sample A, the protein-rich peak C species are contained in fractions 2 and 3. Fraction 2 of sample B and fractions 4 and 5 of both samples have similar M_r distributions and consist mainly of the protein-deficient peak A species.

Yariv reagent and immuno-dot blots

Both whole gum samples and the fractions derived from them were tested for the presence of AGP on dot blots using β-glucosyl Yariv reagent (Table 5). In both cases, the whole gum and all their associated fractions bound the reagent indicating that they all contained AGP. The amount of AGP present in the fractions of sample A as judged by the intensity of the binding to the reagent decreased in the order F1 < F5 < F2 < F4 < F3. For the fractions derived from sample B, the order of affinity for β-glucosyl Yariv reagent was F1 < F6 < F5 < F2 < F4 < F3. These results are of interest in that, as shown in Table 2, the mol % of arabinose and galactose for both samples and their associated fractions are all very similar. It is possible, therefore, that the observed differences in Yariv reagent-binding are due to the different amino acid compositions of the fractions, particularly the variation in the amino acids commonly associated with AGPs, namely Ala, Ser and Hyp (Tables 3 and 4).

The same fractions were also screened against a panel of anti-AGP monoclonal antibodies (Table 6). In these experiments, sample A and all the fractions derived from it were shown to contain the AGP epitopes recognized by the complete panel of antibodies. Fraction 1 reacted most strongly with all seven antibodies which supports the results obtained for the Yariv reagent dot blots. When sample B and its fractions were tested, all six fractions were shown to contain the epitopes recognized by the antibodies JIM 4, JIM 8, JIM 13, JIM 16 and MAC 207. As for sample A, the fractions which reacted most strongly with all seven antibodies, namely fractions 1 and 6, also demonstrated strong binding to β-glucosyl Yariv reagent. The apparent lack of interaction of the whole gum with JIM 14 and JIM 15, in conjunction with the reaction of fractions 1, 2, 3 and 6 with these antibodies, indicates that the AGP epitopes recognized by these antibodies present in the whole gum have been concentrated in these fractions.

Table 3. Amino acid analysis of gum arabic sample A and IEC fractions

Amino acid	Whole gum		Fraction 1		Fraction 2		Fraction 3		Fraction 4		Fraction 5	
	nmol mg^{-1}	Res./1000 Res.	nmol mg^{-1}	Res./1000 Res.	nmol mg^{-1}	Res./1000 Res.	nmol mg^{-1}	Res./1000 Res.	nmol mg^{-1}	Res./1000 Res.	nmol mg^{-1}	Res./1000 Res.
Hyp	39	252	64	336	13	55	6.2	71	6.2	259	12	358
Asp	9	58	4.2	22	26	110	10	115	1.2	50	1.0	30
Thr	12	77	17	89	14	59	4.6	53	1.5	63	2.1	63
Ser	25	161	35	184	22	93	8.5	97	3.0	125	4.3	128
Glu	6.2	40	4.2	22	21	89	8.4	96	1.4	59	1.4	42
Pro	11	71	14	74	14	59	5.6	64	1.6	67	2.4	72
Gly	8.4	54	8.2	43	21	89	7.7	88	1.3	54	1.5	45
Ala	4.4	28	5.1	27	19	81	5.4	62	1.1	46	1.3	39
Cys	—	—	—	—	—	—	—	—	—	—	—	—
Val	5.6	36	3.7	19	17	73	6.8	78	0.9	39	0.9	28
Met	0.3	2	0.6	2	22	10	0.3	3	0.1	6	0.1	4
Ile	1.8	12	1.5	8	5.7	24	1.7	20	0.3	14	0.4	12
Leu	12	78	15	79	20	85	7.7	88	1.8	75	2.1	63
Tyr	1.6	11	1.8	9	3.2	14	0.8	9	0.3	11	0.4	12
Phe	4.7	30	3	16	14	59	6.3	72	0.6	27	0.7	20
His	8.6	56	11	58	8.9	38	3.6	41	1.6	67	1.9	57
Lys	4	26	1.3	7	10	42	2.9	33	0.6	25	0.7	22
Arg	1.3	8	0.9	5	4.7	20	0.8	10	0.31	13	0.3	6
% Nitrogen	0.30		0.32		0.43		0.17		0.05		0.06	
NCF	6.70		6.65		6.55		6.80		6.53		7.50	
% Protein	2.0		2.15		2.8		1.12		0.31		0.46	
% Total protein	—		76.7		7.5		9.8		3.3		2.6	

Table 4. Amino acid analysis of gum arabic sample B and IEC fractions

Amino acid	Whole gum		Fraction 1		Fraction 2		Fraction 3		Fraction 4		Fraction 5		Fraction 6	
	nmol mg^{-1}	Res./1000 Res.	nmol mg^{-1}	Res./1000 Res.	nmol mg^{-1}	Res./1000 Res.	nmol mg^{-1}	Res./1000 Res.	nmol mg^{-1}	Res./1000 Res.	nmol mg^{-1}	Res./1000 Res.	nmol mg^{-1}	Res./1000 Res.
Hyp	50	279	70	323	32	316	21	154	19	290	18	302	29	136
Asp	7.6	42	6.7	31	3.3	33	9.6	70	3	46	2.3	39	14	66
Thr	15	84	19	88	8.2	81	9.5	70	3.9	60	3.1	53	14	66
Ser	30	167	35	162	15	148	13	95	8.9	136	7.1	119	32	150
Glu	6.3	35	5.9	27	2.5	25	10	73	3.7	56	3.3	55	17	80
Pro	15	84	18	83	8.1	80	7.8	57	3.9	60	3.3	55	11	52
Gly	8.4	47	9	42	4.3	42	10	73	4.6	70	3.8	64	21	99
Ala	4.1	23	5	23	3.2	32	11	81	2.4	37	2.5	42	13	61
Cys	—	—		—	—	—	—	—	—	—	—	—	0.4	2
Val	5.2	29	4.9	23	2.8	28	6	44	2.3	35	2.1	35	8.4	39
Met	—	—	0.8*	4	0.8*	8	4*	29	1*	15	0.8*	13	3.6	19
Ile	1.7	9	1.9	9	1.7	17	4.1	30	1.2	18	1.2	20	4.4	21
Leu	14	78	16	74	7.2	71	10	73	4.3	66	3.8	64	14	66
Tyr	21.6	9	1.6	7	1.1	11	2.4	18	0.8	12	1.2	20	3	14
Phe	5.1	28	4.3	20	1.6	16	3	22	1.4	21	1.5	25	4.6	22
His	10	56	13	60	6.3	62	6*	44	3.2	49	3*	50	10*	47
Orn	—	—		—	—	—	—	—	—	—	0.9	15	4.5	21
Lys	3.7	21	3.6	17	2.2	22	5.3	39	1.2	18	1.1	18	5.5	26
Arg	1.5	8	1.8	8	1	10	3.6	26	1.7	11	0.7	12	3.6	17
Nitrogen	0.35		0.38		0.19		0.24		0.10		0.10		0.38	
NCF	6.8		6.62		6.58		5.27		5.45		4.77		5.4	
% Protein	2.35		2.51		1.23		1.26		0.52		0.49		2.03	
% Total protein			76.9		20.3		1.20		0.70		0.5		0.4	

*Co-elutes with unknown.

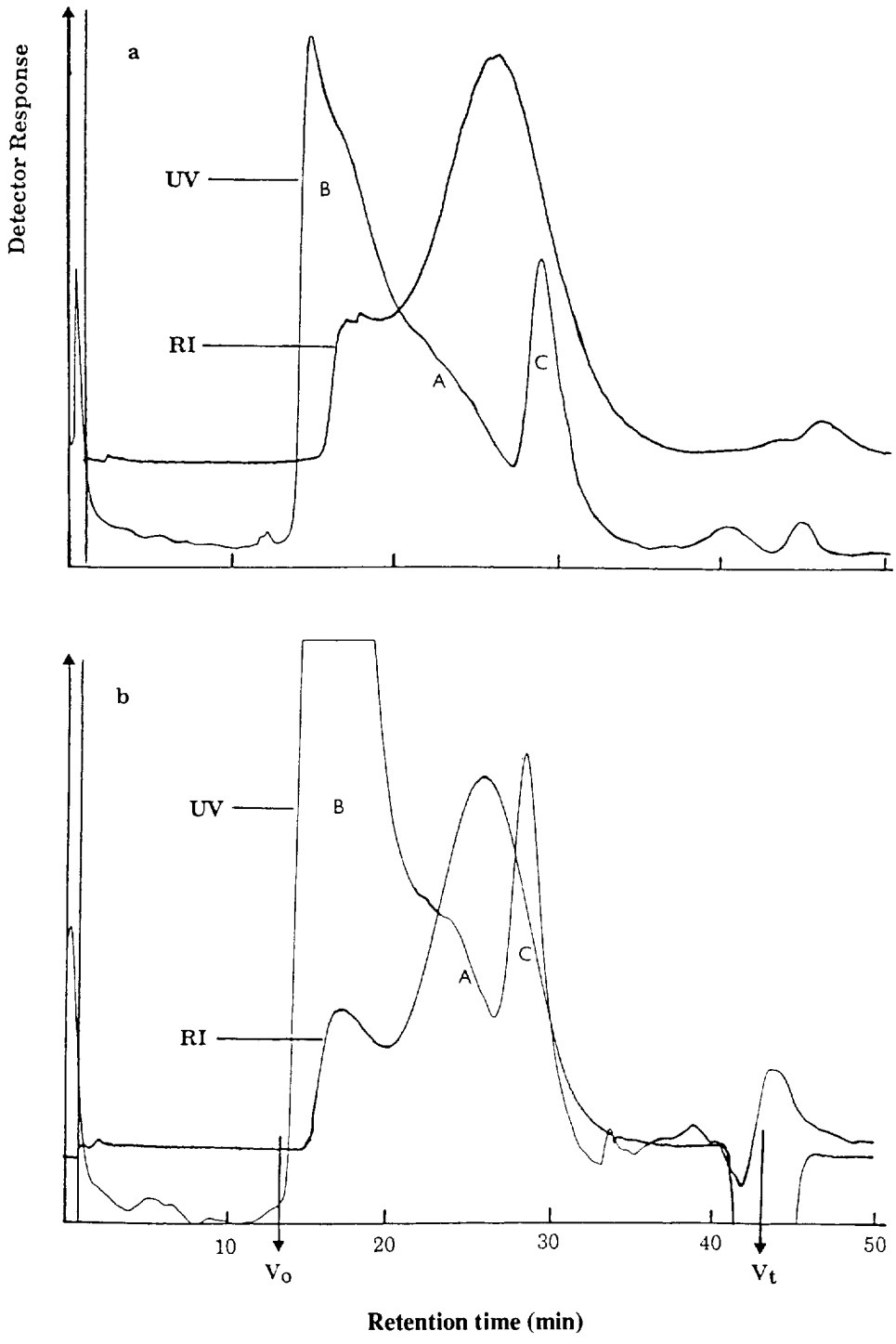

Fig. 1(a) Chromatogram of gum arabic sample A separated on a Superose 6 HR column. Mobile phase, 0.5 M NaCl; sample volume, 100 μl; sample concentration, 1% w/v in mobile phase; flow rate, 0.5 ml min^{-1}; dual detection at A_{206} nm (---) and by RI (—). (b) Chromatogram of gum arabic sample B. Conditions as for Fig. 1a.

DISCUSSION

The current study has demonstrated that gum arabic can be separated into five or six fractions using ion-exchange chromatography. The fractions obtained, however, were found to be polydisperse rather than single M_r species clearly demonstrating, therefore, the extremely heterogeneous nature of the gum. In previous papers [7, 11], we have shown that the gum can be readily separated into three fractions using hydrophobic affinity chromatography and that these fractions are also polydisperse. Interestingly, the fractions obtained using these two

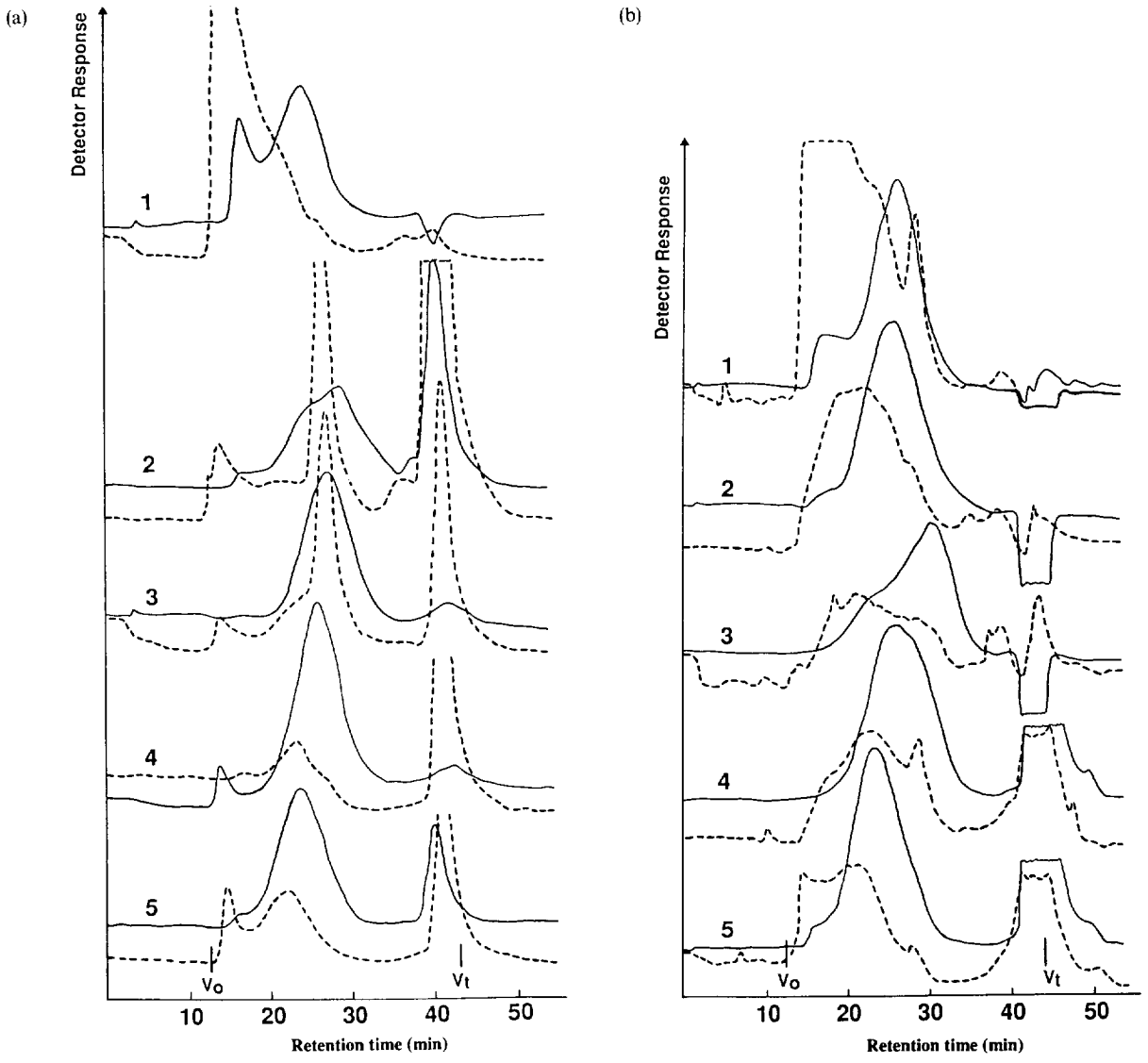

Fig. 2. (a) Chromatograms of gum arabic (sample A) fractions prepared by ion-exchange chromatography on DEAE-cellulose. Conditions as for Fig. 1. (b) Sample B; legend as for Fig. 1a.

Table 5. Interaction of β-glucosyl Yariv regent with gum arabic samples A and B and fractions thereof

	Whole gum	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6
Sample A	+ + + +	+ + + + +	+ + +	+	+ +	+ + + +	−
Sample B	+ + + +	+ + + + +	+ +	+	+ +	+ + +	+ + + + +

Table 6. Interaction of anti-AGP monoclonal antibodies with gum arabic fractions

Antibody	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Whole gum
Mac 207	+ + +	+ +	+ +	+ +	+ + + +	+ + + +	+ + +
JIM 4	+ +	+	+ +	+ +	+ + +	+ + +	+ +
JIM 8	+ + +	+ +	+ +	+ +	+ + +	+	+ + + +
JIM 13	+ + + + +	+ + +	+ + +	+ + +	+ + + +	+ +	+ + + + +
JIM 14	+ +	+	+	−	−	+	−
JIM 15	+ +	+	+	−	−	+	−
JIM 16	+ + +	+	+ +	+	+ +	+ +	+ + +

techniques differ significantly. Hydrophobic affinity chromatography separates the gum mainly according to the protein content of the species present and enabled us to isolate several fractions which had a high protein content (10–50%) [7, 11]. Ion-exchange chromatography, on the other hand, separates molecules according to the proportion of anionic groups (presumably carboxylate groups) which interact with the positively charged sites on the cellulose ion-exchange matrix. None of the fractions obtained using this second method have a particularly high protein content. Since the order of elution of the fractions does not follow their glucuronic acid content, it suggests that some groups may be sterically hindered from interacting with the matrix surface. It has been established previously that the glucuronic acid residues are either at the end of the branch chains or one residue inside the chain end. It is highly probable that the in-chain glucuronic acids are unable to interact with the DEAE-cellulose matrix.

This and previous investigations demonstrate that gum arabic is a complex material consisting of a wide range of carbohydrate and glycoprotein/proteoglycan molecular constituents which contain similar proportions of sugars and share common structural features [22]. Variations in the composition of the two samples studied may simply be due to genetic differences between trees from which the samples are taken, or variations in the age of trees from which the gum is collected. It may be, however, that all the various components that make up the gum are present in each sample but are present in various quantities since they are at various stages of biosynthesis or because some protein or carbohydrate may have been upregulated prior to gum collection in response to pathogenic attack or environmental stress. With regard to the latter two explanations, it is of interest to note that all the five major classes of cell wall proteins namely, the extensins, glycine-rich proteins, proline-rich proteins, the Solanaceous lectins and the arabinogalactan-proteins have been shown to play a role in plant defence/wound healing [23, 24]. Moreover, since AGPs have long been shown to be a major component of gum samples [4, 5], and since a number of other as yet unidentified proteins have recently also been shown to be present in the gum [11], it is therefore possible to hypothesize that variations in the levels of these proteins (some of which, e.g. AGPs, bear large carbohydrate moieties) in response to stress stimuli shortly prior to gum collection may explain the observed variation in molecular composition of samples taken from different sources.

In conclusion, it is clear that the eventual elucidation of the biochemical process of gummosis will necessitate characterization of all of the individual molecular species present within the gum. We are currently developing multi-step fractionation procedures to enable us to isolate the individual components of the gum and to initiate the molecular analysis of these molecules.

EXPERIMENTAL

Materials. Two authentic samples of gum arabic (A and B) were studied. Sample A was a kibbled sample of gum arabic originating from Sudan (Kordofan Province) and supplied by Agrisales Ltd. Sample B was collected by one of us (PAW) from *A. senegal* trees growing in the clay plain El-Dali region of north-eastern Sudan. The nodules collected were amber yellow in colour, *ca* 2–3 cm in diameter and contained very little extraneous material. Moisture contents of samples A and B were 14.9% and 16.0%, respectively.

Diethylaminoethyl cellulose (DEAE-cellulose, DE 52; preswollen granules) anion-exchange resin was a gift from Whatman Specialty Products Ltd.

Fractionation on DEAE-cellulose. A chromatography column (26 × 700 mm) packed with DEAE-cellulose in 1 mM Phosphate buffer (pH 7) was washed 2–3 times with buffer and left to settle overnight at room temp. Gum (20 g) was dissolved in 200 ml 1 mM Phosphate buffer, pH 7, and was passed down (72 ml hr^{-1}). The eluent was monitored by UV absorption (A_{214} nm, A_{280} nm) using a diode array detector and frs (10 ml) collected. The gum which adsorbed to the gel matrix was desorbed by stepwise increases in the ionic strength of the elution buffer using NaCl (0.04–0.29 M). The eluted frs were pooled, extensively dialysed against distilled H_2O and freeze-dried.

Specific rotation. The $[\alpha]_D$ of the whole gum and frs (1% w/v) was determined by polarimetry as previously described [6, 7].

Sugar analysis. Gum samples and frs were hydrolysed with H_2SO_4 (10 ml, 4%) at 100° for 4 hr as previously described [6]. Hydrolysates were neutralized with $BaCO_3$ (*ca* 2 g) and filtered (0.45 μm, Millipore). Filtrates were injected (60 μl) into a HPLC systems and sepd on a S5 amino silica column (4 × 250 mm; Phase Separations Ltd) using a mobile phase of MeCN–H_2O (3:1). The sugar composition of the samples was determined from standard curves of relative peak height against concn.

Estimation of glucuronic acid. The glucuronic acid content of samples A and B and their frs was determined using the *m*-hydroxydiphenyl–H_2SO_4 method [25]. D-Glucuronolactone was used as standard.

Amino acid analysis. Amino acid analysis was carried out by Alta Bioscience Ltd and total N, N conversion factors and protein content of the samples calcd.

M_r *distribution.* The M_r distribution of samples was determined by gel permeation chromatography on a Superose 6 HR FPLC column (10 × 300 mm, Pharmacia) as previously described [21, 26]. Samples (100 μl, in 0.5 M NaCl) were injected and eluted with a mobile phase of 0.5 M NaCl at a flow rate of 0.5 ml min^{-1}. The eluent was detected by UV absorption at 206 nm and by differential refractometry.

Yariv reagent dot blots. Yariv reagent dot blots were performed as previously described [11].

Immuno-dot blots. Immuno-dot blots were performed as previously described [11] using a panel of anti-AGP monoclonal antibodies which bind to carbohydrate epitopes present on these molecules [27].

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REFERENCES

1. Street, C. A. and Anderson, D. M. W. (1983) *Talanta* **30**, 887.
2. Churms, S. C., Merrifield, E. M. and Stephen, A. M. (1983) *Carbohydr. Res.* **123**, 267.
3. Anderson, D. M. W., Hirst, E. L. and Stoddart, J. F. (1966) *J. Chem. Soc. (C)*, 1959.
4. Fincher, G. B., Stone, B. A. and Clarke, A. E. (1983) *Ann. Rev. Plant Physiol.* **34**, 47.
5. Akiyama, Y., Eda, S. and Kato, K. (1984) *Agric. Biol. Chem.* **48**, 235.
6. Randall, R. C., Phillips, G. O. and Williams, P. A. (1988) *Food Hydrocolloids* **2**, 131.
7. Randall, R. C., Phillips, G. O. and Williams, P. A. (1989) *Food Hydrocolloids* **3**, 65.
8. Connolly, S., Fenyo, J.-C. and Vandevelde, M. C. (1987) *Food Hydrocolloids* **1**, 477.
9. Connolly, S., Fenyo, J.-C. and Vandevelde, M. C. (1988) *Carbohydr. Polym.* **8**, 23.
10. Qi, W., Fong, C. and Lamport, D. T. A. (1991) *Plant Physiol.* **96**, 848.
11. Osman, M. E., Menzies, A. R., Williams, P. A., Phillips, G. O. and Baldwin, T. C. (1993) *Carbohydr. Res.* **246**, 303.
12. Jones, J. K. N. and Smith, F. (1949) *Advan. Carbohydr. Chem.* **4**, 243.
13. Anderson, D. M. W. and Dea, I. C. M. (1986) *Carbohydr. Res.* **6**, 104.
14. Ghosh, S. S. and Purkayastha, S. K. (1962) *Indian Forester* **88**, 92.
15. Joseleau, J.-P. and Ullmann, G. (1983) *Bull. IGSM* **13**, 46.
16. Joseleau, J.-P. and Ullmann, G. (1990) *Phytochemistry* **29**, 3401.
17. Jermyn, M. A. (1962) *Aust. J. Biol. Sci.* **5**, 787.
18. Anderson, D. M. W., Brown Douglas, D. M., Morrison, N. A. and Wang Weiping (1990) *Food Addit. Contam.* **7**, 303.
19. FAO (1990) Food and Nutrition Paper 49, FAO, Rome, pp. 23–25.
20. Anderson, D. M. W. (1986) *Food Addit. Contam.* **3**, 231.
21. Osman, M. E., Williams, P.A., Menzies, A. R. and Phillips, G. O. (1993) *J. Agric. Food Chem.* **41**, 71.
22. Williams, P. A., Phillips, G. O. and Stephen, A. M. (1990) *Food Hydrocolloids* **4**, 305.
23. Baldwin, T. C., McCann, M. C. and Roberts, K. (1993) *Plant Physiol.* **103**, 115.
24. Showalter, A. M. (1993) *Plant Cell* **5**, 9.
25. Blumenkrantz, N. and Asboe-Hansen, G. (1973) *Anal. Biochem.* **54**, 484.
26. Menzies, A. R., Osman, M. E., Phillips, G. O. and Williams, P. A. (1992) in *Gums and Stabilizers for the Food Industry* 6 (Phillips, G. O., Wedlock, D. J. and Williams, P. A., eds), pp. 507–512. Oxford University Press, Oxford.
27. Knox, J. P., Linstead, P. J., Peart, J., Cooper, C. and Roberts, K. (1991) *Plant J.* **1**, 317.