Polymer Bulletin 21, 517-521 (1989)

Polymer Bulletin

Meaning of molecular weight measurements of gum arabic

S. Duvallet, J.C. Fenyo*, and M.C. Vandevelde

Laboratoire des Echanges Cellulaires, SCUEOR, URA CNRS n° 203, Faculté des Sciences et Techniques, Université de Rouen, BP 118, F-76134 Mont-Saint-Aignan Cedex, France

SUMMARY

The weight-average M_w and number-average M_n molecular weights of gum arabic are identical after a proteolysis treatment with pronase. The value (1.8×10^5) is closed from M_n early reported in the literature whereas M_w before treatment are dispersed for a large lot of samples up to more 10^6. This can be interpreted by the "wattle blossom" model for which some homogeneous chains of molecular weight c.a. 2.10^5 are still linked to a protein core, the crude gum being a mixture of this complex and free chains.

INTRODUCTION

Molecular weights of Acacia senegal (gum arabic) show wide variations which can be mainly attributed to the method used for the determinations and the heterogeneity of samples (GLICKSMAN and SAND 1973).
Valuable examination of the literature can be carried out only by taking into account the specificity and the limits of the experimental method. Number-average molecular weights (M_n) by osmometry are reported around 200,000 (OAKLEY 1935, 1936, 1937). By ultracentrifugal studies, SAVERBORN (1944) gives a molecular weight of 300,000. Larger values are usually measured by light-scattering for the weight-average molecular weights (M_w) up to 1.0×10^6 (VEIS and EGGENBERGER 1954), but generally around M_w = 580,000 (MUKHERJEE and DEB 1962, ANDERSON et al. 1967). The M_w of a large batch of well identified nodules are dispersed from 4.0×10^5 to 2.2×10^6 (VANDEVELDE and FENYO 1985). SWENSON et al. (1968) found M_n = 250,000 and M_w = 365,000 on the same sample and ACHARAYA and CHATTORAJ (1975) have reported data depending on the nature of the media, temperature and pH.
A better understanding of the polydispersity defined as a distribution in molecular weight but not related to chemical heterogeneity was achieved by fractional precipitation.
ANDERSON and STODDART (1966) have estimated molecular weights of the fractions (coacervation by sodium sulphate) by size-exclusion chromatography on Bio-Gel P300 (Bio-Rad) columns

*To whom offprint requests should be sent

calibrated with dextran fractions of known M_n. ALAIN and Mc MULLEN (1985) have fractionated gum arabic with n-propyl alcohol and established a Mark-Houwink equation by tonometric and viscosity measurements.
However, the more confident results are afforded by light-scattering measurements of the molecular weights on fractions obtained by fractional precipitation (ANDERSON and RAHMAN 1967) or recently by size-exclusion chromatography on well-suited Sephacryl S-400 and S-500 gels (Pharmacia) (VANDEVELDE and FENYO 1985). The Mark-Houwink relations of these two last publications between the intrinsic viscosity (in 1M NaCl) and M_w are in fair agreement.
As usual for natural products, some applications of gum arabic are known to vary with its origin, ageing effects and treatments, which cannot be explained only by polydispersity. However, no clear connection has been still established between the molecular weights and these effects. For this reason, the molecular weight is not a characteristic in control.
The physical and chemical heterogeneity of gum has been recognized for a long time (LEWIS and SMITH 1957, JERMYN 1962, ANDERSON et al. 1967, FUJIWARA and AKAI 1982), but it was only recently evidenced that gum arabic is an arabinogalactan-protein complex (AGP) (AKIYAMA et al. 1984, ANDERSON et al. 1985, ANDERSON and Mc DOUGALL 1987). In this sense, we have proposed a new approach of the macromolecular structure based on a "wattle blossom" model. The proteolysis by pronase, a broad spectrum proteinase produced by Streptomyces griseus, of a lot of samples of Acacia senegal of various origin and story leads always to units of M_w c.a. 200,000, the properties of them in sugar composition, equivalent weight and specific rotation being unchanged. The origin of gum heterogeneity is thus considered to be due to a variable number of these sub-units linked to the protein core (CONNOLLY et al. 1987a,b 1988).
To confirm this model which could explain the apparent polydispersity, it appeared interesting to compare number-average and weight-average molecular weights on the same sample before and after the proteolysis treatment.
The results of these experiments are reported in the present paper.

EXPERIMENTAL

The sample was an individual nodule of Acacia senegal collected in the province of Kordofan (Republic of Sudan) of specific rotation $[\alpha]_{25}^{589} = -30,0°$ in 1M NaCl.
Its purification, preparation of solutions, methods for size-exclusion chromatography, viscometry, low-angle laser light-scattering and enzymatic treatment were just performed as described (CONNOLLY et al. 1987a,b 1988).
A 501 Hewlett-Packard (U.S.A.) high speed membrane osmometer was used to determine the number-average molecular weight at 37,0° C. Diachema A.G. (Switzerland) cellulose membranes (nominal cut off 5,000) were preliminary treated in hot water (60° C) for 15 mn, then in a methanol - water (v/v) mixture in the same conditions and kept in this media. The solvent for

osmometry (aqueous 0,01M NaCl) was degassed prior to use. Gum arabic solutions were equilibrated overnight at 40° C before measurements. M_n was calculated as usual from :

$$1/M_n = (1/RT)x\lim(\pi/C) \text{ when } C->0$$

where π is the excess of osmotic pressure (in cm) between solution and solvent and C the concentration in $g.l^{-1}$.
Due to the high molecular weight of gum arabic for such a technique and the aqueous media needed, accurate measurements require relatively large concentrations when we compared with light-scattering and were repeated until reproductive results were obtained as shown in Fig.1 after the equilibrium was reached in 40 mn.

RESULTS AND DISCUSSION

The elution profile of the sample on Sephacryl S-400 gel and the macromolecular data were compared before and after pronase treatment (Fig. 2 and Table 1). We have previously reported that after proteolysis the rather broad system of several peaks is resolved in a single symmetrical peak. The weight-average molecular weight decreases dramatically from $7.2x10^5$ to $1.8x10^5$ whereas M_n is not affected by proteolysis. These M_n values are also consistent with those previously reported by OAKLEY (1935,1936,1937) and SWENSON et al. (1968). It is also striking that the M_w and $[\eta]$ after pronase treatment are very closed from those observed for fraction C which represents at least 70% of the material (VANDEVELDE and FENYO 1985).

Table 1 - Macromolecular parameters of the sample

	Crude gum	Treated
M_w (a)	$7.2x10^5$	$1.8x10^5$
M_n (b)	$1.9x10^5$	$1.8x10^5$
$[\eta]$ (c)	21.8	11.4

(a): light-scattering determination in 1M NaCl at 25.0° C
(b): osmometry determination in 0.01M NaCl at 37.0° C
(c): $ml.g^{-1}$, direct determination in 1M NaCl at 25.0° C

These new results confirm the "wattle blossom" model for gum arabic. M_n can be considered as an intrinsic property as specific rotation or equivalent weight while M_w is the reflect of heterogeneity restricted to the protein core which links some homogeneous and well defined chains only composed of sugars and of polydispersity index 1 if the notion has still a sense in this case.
It has not yet been clearly evidenced if these chains are composed of a sub-repetitive unit of lower molecular weight (CHURMS et al. 1983, DEFAYE and WONG 1986) but our results are compatible with the structure proposed by STREET and ANDERSON (1983), the calculated molecular weight of their highly branched pattern built after the observation of effect of several sequential Smith-degradation being $1.5x10^5$.
On the other hand, if the weight-average molecular weight is not representative of Acacia senegal, the role of the proteinaceous component on the emulsifying properties of gum

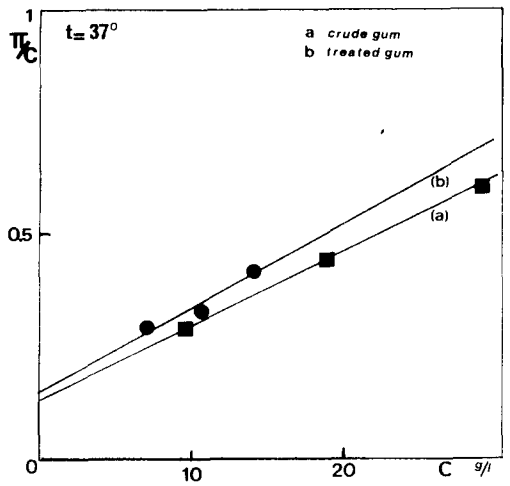

Fig. 1. Reduced osmotic pressure in 0,01M NaCl

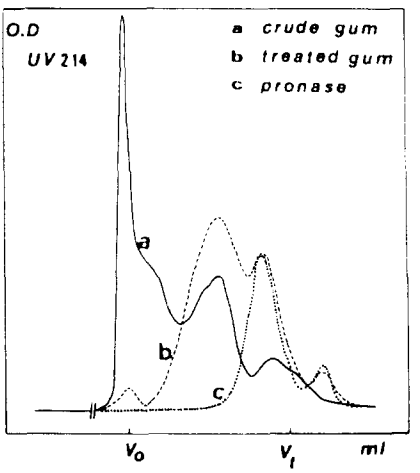

Fig. 2. Size-exclusion chromatography on Sephacryl S-400 gel. Column 2.5x59 cm. Eluant 1M NaCl, 130 ml.h^{-1}. Injection of 5ml at 3% .

arabic was recently evidenced (SNOWDEN et al. 1987, RANDALL et al. 1988). The authors have shown that stable emulsions could not be produced using enzyme-degraded gum. This confirms that the protein which plays probably an important role in the biosynthesis process is also active in the use of the gum.

REFERENCES

ACHARYA, L. and CHATTORAJ, D. K.:Ind.J.Chem.13,569(1975)
AKIYAMA, Y., EDA, S. and KATO, K.:Agric.Biol.Chem.1,235(1984)
ALAIN, M. and Mc MULLEN,J. N.:Int.J.Pharm.23,265(1985)
ANDERSON, D. M. W. and STODDART, J.F.:Carbohydr.Res.2,104(1966)
ANDERSON, D .M. W. and RAHMAN, S.:Carbohydr.Res.4,298(1967)
ANDERSON, D. M. W., HIRST, E., RAHMAN, S. and STAINSBY, G.: Carbohydr.Res.3,308(1967)
ANDERSON, D. M. W., HOWLETT, J. F. and Mc NAB, C. G. A.:Food Addit.Contam.2,159(1985)
ANDERSON, D. M. W. and Mc DOUGALL, F. J. :Food Addit.Contam.4, 125(1987)
CHURMS, S. C., MERRIFIELD, E. H. and STEPHEN, A. M. :Carbohydr.Res.123,267(1983)
CONNOLLY, S., FENYO, J. C. and VANDEVELDE, M. C.:Food Hydrocolloids 1(5-6),477(1987)
CONNOLLY, S., FENYO, J. C. and VANDEVELDE, M. C.:C.R.Soc.Biol. Fr.181,683(1987)
CONNOLLY, S., FENYO, J. C. and VANDEVELDE, M. C.:Carbohydr. Polym.8,23(1988)
DEFAYE, J. and WONG, E.:Carbohydr.Res.150,221(1986)
FUJIWARA, T. and AKAI, K.:Carbohydr.Res.101,295(1982)

GLICKSMAN, M. and SAND, R. E.:in Industrial gums, Whistler, R. L.(ed.), New York: Academic Press 1973,p. 207
JERMYN, M. A.:Aust.J.Biol.Sci.15,787(1962)
LEWIS, B. A. and SMITH, F.:J.Am.Chem.Soc.79,3929(1957)
MUKHERJEE, S. N. and DEB, S. K.:J.Ind.Chem.Soc.39(12),823(1962)
OAKLEY, H. B.:Trans.Faraday Soc.31,136(1935)
OAKLEY, H. B.:Trans.Faraday Soc.32,1360(1936)
OAKLEY, H. B.:Trans.Faraday Soc.33,372(1937)
RANDALL, R.C., PHILIPS, G.O. and WILLIAMS, P.A.:Food Hydrocolloids 2(2),131(1988)
SAVERBORN, H.:in Svedberg memorial volume, Uppsala: Almquist and Wiksell 1944, p. 508
SNOWDEN, M. J., PHILIPS, G.O. and WILLIAMS, P.A.:Food Hydrocolloids 1(4),291(1987)
STREET, C.A. and ANDERSON, D. M. W.:Talanta 30(11),887(1983)
SWENSON, H.A., KAUSTINEN, H.M., KAUSTINEN, O.A. and THOMPSON, N. S.:J.Polym.Sci.Part A-2(6),1593(1968)
VANDEVELDE, M. C. and FENYO, J. C.:Carbohydr.Polym.5,251(1985)
VEIS, A. and EGGENBERGER, D. N.:J.Am.Chem.Soc.76,1560(1954)

Accepted March 2, 1989 C