Macromolecules

Volume 2, Number 1 January-February, 1969

The Unperturbed Dimensions of Sodium Carboxymethylamylose

David A. Brant and Byung Kwon Min

Department of Chemistry, University of California, Irvine, California 92664.
Received August 23, 1968

ABSTRACT: Sodium carboxymethylamylose, degree of substitution (DS) = 0.55, has been prepared and fractionated according to molecular weight by gel permeation chromatography. Intrinsic viscosities, osmotic molecular weights, and second osmotic virial coefficients have been determined for the fractions and the bulk polymer in 0.5 *M* aqueous NaCl at pH 8.0 and 35°. Polymer chain dimensions, unperturbed by volume exclusion, have been calculated from the experimental results using the method of Fox and Flory; the expansion factor was evaluated following recent suggestions of Berry. The experimental characteristic ratio $C_\infty = 10$, and this is approximately twice the value found for native amylose in neutral aqueous media. It is concluded that the conformation of sodium carboxymethylamylose in the present medium resembles that of aqueous unsubstituted amylose but that the proportion of helical units is smaller in the derivative owing to steric disruptions of the helical conformation precipitated by the substituent group.

Investigations seeking to establish the connection between polymer backbone structure and chain conformation in dilute solution have had gratifying success in recent years with the advent of improved theoretical methods for dealing with these problems.[1] Yet, despite the biological and commercial significance of polymers of the polysaccharide class, little progress has been made in correlating solution properties with details of the skeletal characteristics of these molecules. Although the structural complexity of the polysaccharides presents formidable obstacles, the absence of consistent experimental values for the unperturbed spatial dimensions imposes at least as great an impediment to progress toward this end. Indeed, in some respects the structural features render theoretical treatment of these molecules significantly less tedious than for simpler polymers, *e.g.*, polyethylene, to the extent that interposition of rigid anhydro sugar rings between the oxygens of the glycosidic bridges serves to render rotations about neighboring pairs of glycosidic bonds mutually independent. Analysis of the conformational statistics in terms of a chain of independent virtual bonds is then possible, following methods developed to treat chains of peptide homopolymers and copolymers.[2,3]

Chief among the problems for determination of unperturbed dimensions of the more common polysaccharides, *e.g.*, cellulose and amylose, has been the high crystalline melting points of many of these species. This results in a paucity of θ solvents and, indeed, of solvents in general, in which to carry out the required measurements. Moreover, efficient molecular weight fractionation by classical means is seriously compromised by the high crystallinity. Consequently, results of many experimental investigations are burdened with uncertain corrections for molecular weight heterogeneity and solvent nonideality. These experimental difficulties must be successfully dealt with and reliable unperturbed chain dimensions determined before appreciable progress may be had in interpreting chain dimensions in terms of backbone structural features. It is within the context of efforts to establish a general correlation of polysaccharide structure and solution properties that the experimental results presented here are offered.

Among the several readily available polysaccharides for which definitive experimental solution properties are lacking, interest in the solution properties of amylose and its derivatives is heightened by evidence[4,5] for significant retention in dilute aqueous solution of the helical structure characteristic of the crystalline material.[6] Hydrodynamic data, however, clearly preclude the existence in solution of rigid helical molecules,[7]

(1) P. J. Flory, "Statistical Mechanics of Chain Molecules," Interscience Publishers, New York, N. Y., 1968.
(2) D. A. Brant and P. J. Flory, *J. Amer. Chem. Soc.*, **87**, 2791 (1965).
(3) W. G. Miller, D. A. Brant, and P. J. Flory, *J. Mol. Biol.*, **23**, 67 (1967).
(4) J. Szejtli and S. Augustat, *Staerke*, **18**, 38 (1966); J. Szejtli, S. Augustat, and M. Richter, *Biopolymers*, **5**, 5, 17 (1967).
(5) C. M. Paulson, Ph D. Thesis, University of California, Berkeley, Calif., 1955.
(6) R. H. Marchessault and A. Sarko, *Advan. Carbohydrate Chem.*, **22**, 421 (1967).
(7) W. Banks and C. T. Greenwood in "Conformation of Biopolymers," Vol. II, G. N. Ramachandran, Ed., Academic Press, New York, N. Y., 1967. p 739.

and a broken or jointed helical conformation provides the most satisfactory model for dissolved amylose in neutral aqueous media.[4,8] Despite the well-known instability of neutral aqueous amylose solutions with respect to crystalline phase separation (retrogradation), an extensive body of evidence has been amassed to suggest that such systems may satisfy the conditions of solvent ideality required for the covolume of the polymer chains to vanish. Thus mutually consistent unperturbed dimensions for unsubstituted amylose have been evaluated from the data of several workers on the presumption that θ conditions obtain near 25° in neutral aqueous solution.[7,9] Reasons for caution in accepting as definitive these superficially satisfying results have already been cited by Rao and Foster[8] and by Banks and Greenwood,[7] who note the negligible temperature coefficient of the intrinsic viscosity and the failure to observe reversible phase separation as temperature is varied in these systems. Other thermodynamic evidence for ideality is likewise inconclusive; although a vanishing second osmotic virial coefficient has been reported,[10,11] other workers have described conflicting results.[12]

In seeking a suitable system for experimental characterization of amylose, attention is directed toward derivatives of the native material as a means to extend the range of possible solvents. Wider latitude in the choice of solvent not only facilitates selection of suitable conditions for reliable determination of unperturbed dimensions but also makes available alternative means for securing efficient molecular weight fractionation. Systems susceptible to preparative scale gel permeation chromatography are particularly to be sought. On the other hand, perturbations of the skeletal characteristics of the native material precipitated by the chemical modification must be rigorously avoided, or the aforementioned advantages deriving from such modification are without benefit for the purposes at hand.

Polyelectrolyte ether derivatives at low degrees of substitution appear to satisfy these criteria. At low degrees of substitution reaction should be confined principally to the primary alcohol at the C_6 position of the anhydroglucose ring.[13] The ether linkage is not bulky, and the availability of several conformers for the C_5–C_6 bond serves to reduce the influence of substitution at C_6. Solubility in aqueous as well as organic media can be ensured with these derivatives. Of particular importance, stable aqueous solutions are subject to gel permeation chromatography on relatively inexpensive hydrophylic gels. Efficient molecular weight fractionation may thereby be achieved with negligible intrusion of competing processes which may accompany the fractional crystallizations frequently used. Finally, the polyelectrolyte character of the chain renders it susceptible to manipulation of the chain conformation through variation of the charge density and ionic strength; both positively and negatively charged ether derivatives are available, and many of these are weak electrolytes which can be studied in ionized and un-ionized states. The recent report by Rao and Foster[14] of studies on carboxymethylamylose (CMA) suggested that this derivative would be appropriate to initiate our studies, inasmuch as its ability to form the well-known blue complex with iodine verifies its conformational similarity with native amylose. The preparation, molecular weight fractionation, characterization of the fractions, and subsequent evaluation of the characteristic ratio of the mean square unperturbed end-to-end distance are reported in what follows.

Experimental Section

Recrystallization of Amylose. Commercial amylose, marketed under the trade name Superlose (Stein, Hall and Co., Inc., New York, N. Y.), was generously donated by the manufacturer. It was recrystallized as the butanol complex following a modification of the method of Gilbert, *et al.*[15] Thus 50 g of amylose was dissolved in 3 l. of 1 *M* aqueous NaOH. When dissolution was complete, the solution was diluted to 5 l. with water and then neutralized with 1 *M* HCl. The resulting solution was centrifuged immediately for 30 min at 5000 rpm to remove undissolved material. The slightly opalescent centrifugate was warmed to 80° and allowed to react with 260 ml of *n*-butyl alcohol, this quantity of *n*-butyl alcohol having been chosen in accordance with the recommendations of Muetgeert.[16] The solution was allowed to cool over a period of 6 hr to 25°; precipitation of the butyl alcohol–amylose complex commenced at about 50°. The complex was collected by centrifugation and dissolved in 1500 ml of 0.5 *M* NaOH. Removal of *n*-butyl alcohol was accomplished by bubbling N_2 gas through the solution for 30 min while heating in a boiling water bath. A 100-ml sample of the butanol-free solution was dialyzed exhaustively against distilled water. The amylose which precipitated in the dialysis bag was recovered for determination of the "blue value."[17]

Preparation of Sodium Carboxymethylamylose. Saturated NaOH was added to the remaining 1400 ml of solution to bring the concentration of NaOH to 4 *M*. Then 150 g of reagent grade $ClCH_2COOH$ (1.5-fold excess for DS = 1) was added with cooling as required, and the mixture was allowed to react at 55° for 4 hr. The reaction mixture was neutralized with glacial acetic acid, and the sodium carboxymethylamylose (NaCMA) was recovered by exhaustive dialysis and subsequent freeze-drying.

Determination of Degree of Substitution. The equivalent weight, M_{CMA}, of the protonated product was determined by potentiometric titration with 1.000 *M* NaOH of 10 ml of pure aqueous CMA solution (*ca.* 0.5% polymer by weight) prepared from the salt by cation exchange. Titrant was

(8) V. S. R. Rao and J. F. Foster, *Biopolymers*, **1**, 521 (1963).
(9) H. L. Griffin, S. R. Erlander, and F. R. Senti, *Staerke*, **19**, 8 (1967).
(10) W. W. Everett and J. F. Foster, *J. Amer. Chem. Soc.*, **81**, 3459 (1959).
(11) W. Banks and C. T. Greenwood, *Macromol. Chem.*, **67**, 49 (1963).
(12) W. Burchard, *ibid.*, **64**, 110 (1963).
(13) This presumption is based on evidence suggesting less steric restriction to substitution at the primary alcohols of sugars and polysaccharides. Both triphenylmethyl ethers and *p*-toluenesulfonate esters are formed preferentially at the primary hydroxyl positions: "The Carbohydrates," Ward Pigman, Ed., Academic Press, New York, N. Y., 1957, Chapters 3 and 7.
(14) V. S. R. Rao and J. F. Foster, *Biopolymers*, **3**, 185 (1965).
(15) L. M. Gilbert, G. A. Gilbert, and S. P. Spragg in "Methods in Carbohydrate Chemistry," Vol. IV, R. L. Whistler, Ed., Academic Press, New York, N. Y., 1964, p 25
(16) J. Muetgeert, *Advan. Carbohydrate Chem.*, **16**, 299 (1961).
(17) G. A. Gilbert and S. P. Spragg in "Methods in Carbohydrate Chemistry," Vol. IV, R. L. Whistler, Ed., Academic Press, New York, N. Y., 1964, p 168.

delivered to a magnetically stirred titration vessel from a calibrated micrometer syringe. Concentrations of CMA solutions were determined by dry weight analysis. Constant dry weight was achieved in the vacuum oven in 4–6 hr in the temperature range 70–95°; higher temperatures degrade the polymer with loss of covalently bound water, whereas lower temperatures do not produce completely dry residues. The degree of substitution is related to the equivalent weight by DS = $162/(M_{CMA} - 58)$.

Molecular Weight Fractionation. Two 2-g samples of the bulk NaCMA, designated S-1 and S-2, were fractionated by gel permeation chromatography on Sephadex G-200 using a Sephadex K 50 column 5 cm in diameter and 90 cm in length (Pharmacia Fine Chemicals, Piscataway, N. J.). Aqueous 0.5% NaCl saturated with chloroform (to prevent microbial degradation) was used as the solvent–eluent system. Polymer samples were applied to the column as 1% solutions, and collected in an automatic fraction collector at a rate of 10 ml hr^{-1}. The effluent was monitored for polymer by following the absorbancy at 600 mμ of the CMA–iodine complex. A weight *vs.* volume distribution curve was thereby constructed, and using this, each sample was separated into four 0.5-g fractions. These were recovered by exhaustive dialysis and freeze-drying, and are designated in the following as S-1-1, S-1-2, etc.

Preparation of NaCMA Solutions for Viscometry and Osmometry. Samples of fractionated NaCMA were dissolved in *ca.* 25 ml of 0.5 *M* NaCl solution saturated with chloroform to make 1–2% stock solutions of NaCMA. Stock solutions were filtered, adjusted to pH 8.0 with 1 *M* NaOH, and dialyzed against *ca.* 1 l. of the solvent (also adjusted to pH 8) for 1 day in a thermostat at 35°. Air was excluded from the dialysis vessel with N_2 gas to protect the unbuffered, alkaline solutions. Following dialysis, the dialyzate solutions were kept under N_2 in 25-ml erlenmeyer flasks covered with rubber septa to facilitate removal by syringe without exposure to air. Dialysis receiving solutions (solvent) were similarly stored. Solutions of the NaCMA fractions of the required concentrations were then prepared by *gravimetric* dilution of the dialyzed stock solutions with dialysis receiving solution. This dilution was always carried out in 5- or 10-ml volumetric flasks, which in effect provides density information of sufficient accuracy to permit subsequent interconversion of weight and volume concentrations. All solution transfers were accomplished by syringe to avoid contact with atmospheric CO_2. Final solutions for viscometry and osmometry were delivered through syringe mounted Type HA Millipore filters (Millipore Filter Corp., Bedford, Mass.). The pH of the final solutions was always within the range 7.7–8.1; consequently the degree of neutralization was in every case equal to unity.

Concentrations of Stock NaCMA Solutions. Since an unknown dilution occurs upon dialysis, NaCMA stock concentrations were determined following the dialysis. Dry weight concentration determinations were employed, taking into account the unequal distribution of diffusable ionic species between dialyzate and receiving solutions which results from the Donnan effect.[18] To pursue this discussion the following quantities are defined: m_p = equivalents of polymeric carboxylate/gram of H_2O; m_- = equivalents of Cl^-/gram of H_2O; m_+ = equivalents of Na^+/gram of H_2O; M_i = equivalent weight of species or component i; $\gamma_\pm$ = mean ionic activity coefficient of dissolved NaCl; W_i = weight of species or component i/gram of H_2O; W = total weight of all solute species/gram of H_2O. A prime designates the polymer-free phase, and unprimed symbols denote the phase containing polymer. The quantities W and W' are experimentally determined by dry weight analysis under conditions described above. The charge and mass balances governing the system are

$$m_+' = m_-' \tag{1}$$

$$m_+ = m_- + m_p \tag{2}$$

$$W' = W_{NaCl}' = m_-' M_{NaCl} \tag{3}$$

$$W = W_{NaCMA} + W_{NaCl} = m_p M_{NaCMA} + m_- M_{NaCl} \tag{4}$$

The quantity sought, W_{NaCMA}, is given by eq 4 as $W_{NaCMA} = W - m_- M_{NaCl}$. The unknown m_- may be calculated by solving eq 5, which results from combination of eq 3 and 4

$$m_-^2\left(1 - \frac{M_{NaCl}}{M_{NaCMA}}\right) + m_-\left(\frac{W}{M_{NaCMA}}\right) - \left(\frac{W'}{M_{NaCl}}\right)^2\left(\frac{\gamma_\pm'}{\gamma_\pm}\right)^2 = 0 \tag{5}$$

with the Donnan condition expressed in eq 6. Under the

$$(m_-'\gamma_\pm')^2 = (m_- + m_p)m_-\gamma_\pm^2 \tag{6}$$

conditions of the present experiments one expects $\gamma_\pm'/\gamma_\pm = 1$ from the data on similar systems.[19] Moreover, W' was virtually identical for all experiments, because of the relative volumes of dialyzate and receiving solutions. The equivalent weight of NaCMA is given by $M_{NaCMA} = (DS)^{-1}[162 + (DS)80]$. To convert the calculated polymer concentration W_{NaCMA} from units of grams per gram of H_2O to units of grams per gram of solution it must be divided by $1 + W$. The gravimetric uncertainty in W_{NaCMA} was reduced to less than 1.0% by determining W and W' using 5-ml samples.

Intrinsic Viscosity Measurements. Measurements were carried out in size 50 Cannon-Ubbelohde viscometers (Cannon Instrument Co., State College, Pa.) in a thermostat at 35 ± 0.01°. Solvent (dialysis receiving solution) flow times were greater than 190 sec, and no kinetic energy corrections were made. Shear rate dependence of the viscosity was not investigated, and Newtonian behavior was assumed. The density difference between solutions and solvent was inconsequential.[20] Solutions were prepared for viscometry as described previously to yield relative viscosities in the range $1.1 < \eta_r < 1.6$, and these solutions were blanketed continuously with N_2 in the solution reservoir of the viscometers. A minimum of three flow-time measurements was made on each solution, and a mean deviation no more than ±0.1 sec from the mean of all measurements was required.

Osmotic Pressure Measurements. A Mechrolab Model 502 membrane osmometer with variable-temperature controller (Hewlett-Packard, F & M Scientific Division, Avondale, Pa.) operating at 35° was employed for the osmotic pressure measurements. Schleicher and Schuell (Keene, N.H.) B-19 membranes were used. The osmotic pressure of each NaCMA fraction was measured at four different concentrations. For each such solution at least three measurements were made, and the average of these, reduced

(18) H. Morawetz, "Macromolecules in Solution," Interscience Publishers, New York, N. Y., 1965, p 340.

(19) Z. Alexandrowicz, *J. Polym. Sci.*, 43, 325, 337 (1960).

(20) H. Eisenberg and E. F. Casassa, *ibid.*, 47, 29 (1960).

TABLE I
INTRINSIC VISCOSITIES, MOLECULAR WEIGHTS, AND SECOND VIRIAL COEFFICIENTS OF NaCMA IN AQUEOUS 0.5 *M* NaCl AT pH 8 AND 35°

Sample	$[\eta]$, dl/g	$M_n \times 10^{-5}$, g	$A_2 \times 10^4$, ml mol/g^2
S-1-1	1.37	2.01 ± 1%	8.0 ± 2%
S-1-3	0.70	0.58 ± 3%	8.0 ± 2%
S-1-4	0.40	0.35 ± 1%	6.5 ± 2%
S-2-1	1.35	1.87 ± 3%	8.8 ± 3%
S-2-2	0.91	0.90 ± 2%	5.6 ± 3%
S-2-3	0.89	0.83 ± 1%	8.0 ± 3%
Bulk	1.22	1.24 ± 2%	9.2 ± 2%

by the reading with solvent (receiving solution) on both sides of the membrane, was recorded as the osmotic pressure of that solution. Repeated readings for solvent against solvent were obtained before and after measurements on each solution, and the average of values so obtained was taken as the solvent reading. In general a mean deviation of ±0.04 cm was deemed satisfactory in establishing an average reading for any sample.

Results

Experimental Data. Recrystallization of the amylose sample as the butanol complex effected significant improvement in its purity; the "blue value"[17] of the recrystallized product was 1.3 as compared with 1.0 for the starting material, Superlose. Recent investigations show that the recrystallization procedure outlined above yields product with an iodine binding capacity of 18.6% by weight, whereas that of the starting material is 17.5%.[21] Freedom of the sample from significant amounts of branched-chain contaminants is thereby ensured. Equivalent weight determinations on the NaCMA establish the degree of substitution to be 0.55 ± 0.03. This 5% uncertainty in DS provokes an uncertainty of ±0.3% in the concentrations of the NaCMA stock solution in addition to the gravimetric uncertainties previously described. Thus concentrations of all solutions are uncertain to the extent of approximately ±1%.

Results of osmotic pressure and viscosity measurements on the bulk NaCMA and six fractions in aqueous 0.5 *M* NaCl at pH 8 and 35° are presented in Table I, where intrinsic viscosities, number average molecular weights, and osmotic second virial coefficients are given. Uncertainties, when indicated, were estimated by drawing reasonable alternatives to the best lines through the experimental data; they therefore reflect only indirectly the concentration uncertainties previously described. A plot of log $[\eta]$ *vs.* log M_n is shown in Figure 1. The point for the fraction of lowest molecular weight, S-1-4, was ignored in drawing the line through the data, this being the sample most likely to deviate from the asymptotic random flight and hydrodynamic behavior; the point for bulk polymer is included only for comparison. The line shown corresponds to the Mark-Houwink equation

$$[\eta] = 2.09 \times 10^{-3} M^{0.53} \quad (7)$$

(21) A. Cesàro, personal communication.

Unperturbed Dimensions. Principal features of the connection between the intrinsic viscosity and coil dimensions of *random flight* polymer chains are well established.[22] Thus when the coil dimensions are described in terms of the mean square separation of chain ends, the intrinsic viscosity in Flory θ solvent media is given by

$$[\eta]_0 = \Phi_0(\langle r^2\rangle_0/M)^{3/2} M_v^{1/2} \quad (8)$$

The ratio $\langle r^2\rangle_0/M$ of mean chain dimensions, unperturbed by long-range volume exclusion, to the molecular weight of the chain is a constant characteristic of the polymer in question for sufficiently long chains, *i.e.*, for chains possessing a Gaussian distribution of end-to-end distances.[23] The hydrodynamic parameter Φ_0 approaches an asymptote as chain length increases,[22] which is predicted[24] to be 2.68×10^{-3} when dimensions are measured in ångströms and intrinsic viscosity in deciliters per gram. This theoretical limiting value has adequate experimental backing, although the molecular weight range required for convergence is dependent upon the characteristics of the polymer chain and is not subject to *a priori* specification.[22] Use of the viscosity average molecular weight M_v of the polymer sample accounts for the effect of molecular weight heterogeneity.[25]

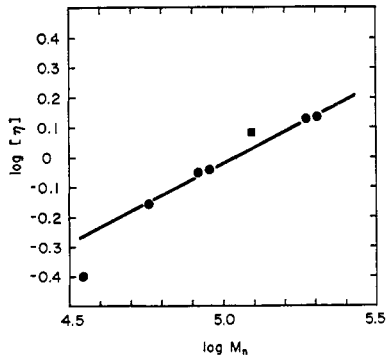

Figure 1. Plot of intrinsic viscosity *vs.* number average molecular weight for sodium carboxymethylamylose fractions in 0.5 *M* aqueous NaCl at pH 8 and 35°. Filled square corresponds to the unfractionated polymer.

To connect chain dimensions and intrinsic viscosities in *good solvents* the uniform expansion approximation of Fox and Flory[26] is frequently employed whereby it is assumed that the intrinsic viscosity increases in proportion to the cube of the expansion of the linear chain dimensions $\alpha = (\langle r^2\rangle/\langle r^2\rangle_0)^{1/2}$. A distinction between

$$[\eta] = \Phi(\langle r^2\rangle_0/M)^{3/2} M_v^{1/2} \alpha^3 \quad (9)$$

Φ and Φ_0 is drawn on theoretical as well as on experimental grounds;[22] in good solvents Φ is frequently

(22) G. C. Berry, *J. Chem. Phys.*, **46**, 1338 (1967).
(23) P. J. Flory and R. L. Jernigan, *ibid.*, **42**, 3509 (1965).
(24) C. W. Pyun and M. Fixman, *ibid.*, **42**, 3838 (1965).
(25) P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953, Chapter 7.
(26) T. G Fox and P. J. Flory, *J. Amer. Chem. Soc.*, **73**, 1904 (1951).

found[27,28] to be near 2.2×10^{-3}, and for polyelectrolytes a still smaller value has been suggested.[29] Strict dependence of $[\eta]$ on the cube of α may in fact be realized only for rather high chain lengths,[22] but the approximation in eq 9 is small in the present instance relative to the problems associated with the determination of α.

It is convenient for the present case to report the unperturbed dimensions in terms of the asymptotic characteristic ratio $C_\infty = (\langle r^2\rangle_0/xl^2)_{x\to\infty}$, where x is the number of bonds of mean length l comprising the chain. The characteristic ratio may be meaningfully compared for members of a series of polymers possessing identical backbone structures but differing in the weight of the repeating unit. Hence, to the extent that the anhydroglucose ring conformation in the amylose backbone is preserved under varying conditions of chemical modification, C_∞ is an appropriate measure of the unperturbed dimensions. Increasingly strong evidence[30-34] for the $C1$ conformation[35] of the amylose glucose moieties leads us to base our evaluation of C_∞ upon a backbone structure comprising exclusively $C1$ glucose residues. (This is not to imply that other anhydroglucose conformations are never represented in amylose and its derivatives but merely to provide a basis for evaluating C_∞ from the experimental results. Minor proportions of other ring conformers actually present will have little effect upon the mean bond length l and, hence, upon C_∞. Theoretical treatment of the conformational statistics must, however, take carefully into account the nature and proportions of all ring conformers.) With this backbone model the conformational statistics of the amylose chain may be treated in analogy with the polypeptides[2,3] in terms of a sequence of identical virtual bonds of fixed length joining the oxygens of the glycosidic bridges. We thus take $l = 4.25$ Å[31] and identify x with the degree of polymerization. The characteristic ratio is given by

$$C_\infty = ([\eta]/\Phi M_v^{1/2}\alpha^3)^{2/3}(M_u/l^2) \qquad (10)$$

where $M_u = 162 + 80(DS)$ is the average weight per monomer unit.

Experiments reported above have yielded $[\eta]$ and M_n, and we assume that the chromatographic fractions employed are sufficiently sharp that $M_v \simeq M_n$; M_v enters eq 10 as the one-third power to render negligible the importance of this approximation in calculating C_∞. For the bulk polymer $M_v = 155{,}000$ as obtained from its intrinsic viscosity and the Mark–Houwink equation established with the fractionated material. We take $\Phi = 2.1 \times 10^{-3}$ as a reasonable estimate for polyelectrolytes in good solvents. Berry[36] has reported that a relationship between α^2 and the second osmotic virial coefficient A_2, derived by eliminating the interaction parameter z of polymer solution theory between first-order theories of inter- and intramolecular polymer interactions, is valid over a wider range of conditions than either of the separate first-order theories. Thus eq 11 is satisfied up to $\alpha^2 = 2.5$ for polystyrene in both

$$\alpha^2 = 1 + \frac{A_2M^{1/2}a_1}{4N(\pi/6)^{3/2}(\langle r^2\rangle_0/M)^{3/2}} + \ldots \qquad (11)$$

decalin and toluene. Here $a_1 = (134/105)$ is a well-known constant from polymer expansion theory and N is Avogadro's number.

The anticipated decrease in A_2 with increasing molecular weight[37] is not apparent in the results reported in Table I. Consequently only the mean value of A_2 for the six fractions was considered significant, and this value, 7.5×10^{-4} ml mol g^{-2}, was used in all subsequent calculations. The value of A_2 obtained for the more heterogeneous bulk material was not included in the average. Since $\langle r^2\rangle_0/M$ appears on the right-hand side of eq 11, iterative procedures must be used to obtain a solution for α^2. As a first approximation to α^2 we have used eq 12 due to Orofino and Flory.[38] Cal-

$$A_2M/[\eta] = 188 \ln [1 + 0.886(\alpha^2 - 1)] \qquad (12)$$

culated values of α^2 from eq 12 and 11 and the resulting values of C_∞ from eq 10 are given in columns 2, 3, and 4 of Table II, respectively. That smaller values of α^2 result from eq 11 than from eq 12 is consistent with the supposition that the smoothed density approximation inherent in the treatment of Orofino and Flory overestimates the molecular expansion.[39]

TABLE II
UNPERTURBED DIMENSIONS AND EXPANSION FACTORS

Sample	α^2 Eq 12	α^2 Eq 11	C_∞	$\langle r^2\rangle_0/xl^2$	$(C_\infty/C_{\infty,f})^{1/2}$
S-1-1	1.90	1.75	8.34	14.6	2.69
S-1-3	1.44	1.26	11.2	14.1	3.12
S-1-4	1.47	1.28	8.96	11.5	2.79
S-2-1	1.84	1.65	8.96	14.8	2.79
S-2-2	1.55	1.34	10.9	14.6	3.07
S-2-3	1.51	1.31	11.2	14.7	3.12
Bulk	1.74	1.54	9.58	14.7	2.89

The average value of C_∞ from Table II is 10.0, calculated with exclusion of the result for fraction S-1-4 which may deviate from asymptotic behavior as evidenced by failure of its intrinsic viscosity to correlate well with those of the higher molecular weight fractions.

(27) S. Newman, W. R. Krigbaum, C. Laugier, and P. J. Flory, *J. Polym. Sci.*, **14**, 451 (1954).
(28) W. R. Krigbaum and D. K. Carpenter, *J. Phys. Chem.*, **59**, 1166 (1955).
(29) H. Eisenberg and D. Woodside, *J. Chem. Phys.*, **36**, 1844 (1962).
(30) V. S. R. Rao and J. F. Foster, *J. Phys. Chem.*, **69**, 636 (1965).
(31) A. Hybl, R. E. Rundle, and D. E. Williams, *J. Amer. Chem. Soc.*, **87**, 2779 (1965).
(32) B. Casu, M. Reggiani, G. G. Gallo, and A. Vigevani, *Tetrahedron*, **22**, 3061 (1966).
(33) V. S. R. Rao, P. R. Sundararajan, C. Ramakrishnan, and G. N. Ramachandran in "Conformation of Biopolymers," Vol. II, G. N. Ramachandran, Ed., Academic Press, New York, N. Y., p 721.
(34) A. Sarko and R. H. Marchessault, *J. Amer. Chem. Soc.*, **89**, 6454 (1967).
(35) R. E. Reeves, *ibid.*, **76**, 4595 (1954).
(36) G. S. Berry, *J. Chem. Phys.*, **44**, 4550 (1966).
(37) Reference 25, Chapter 12.
(38) T. A. Orofino and P. J. Flory, *J. Chem. Phys.*, **26**, 1067 (1957).
(39) P. J. Flory and S. Fisk, *ibid.*, **44**, 2243 (1966).

That the results for the lower molecular weight fractions (except S-1-4) exceed the average whereas those for the higher molecular weight fractions are less than the average is consonant with use of an averaged second virial coefficient. The internal consistency of the results is demonstrated more clearly in column 5 of Table II where the coil dimensions in the present solvent system, $\langle r^2\rangle/xl^2 = \alpha^2 C_\infty$, are shown. In the final column of Table II is reported the square root of the ratio of the observed unperturbed dimensions to those calculated assuming free rotation about the two bonds of the glycosidic linkage. We find[40] the latter quantity $C_{\infty,f} = 1.15$, assuming again the maltose geometry of the α-Schardinger dextrin.[31]

Other procedures for evaluating C_∞ from the experimental data were also investigated. Since the exponent of M in eq 7 is only 0.53, methods which involve extrapolation of intrinsic viscosity *vs.* molecular weight data should be subject to little uncertainty. Following procedures suggested by Berry[22] and by Stockmayer and Fixman,[41] results for C_∞ were obtained which exceed by approximately 40% the average value reported above, providing we again take $\Phi = 2.1 \times 10^{-3}$. The discrepancy between this result based on analysis of intrinsic viscosity *vs.* molecular weight data alone and the prior result obtained utilizing thermodynamic measures of the excluded volume expansion, *i.e.*, A_2, is not easily reconciled. In fact this discrepancy is in the direction contrary to that usually observed when the two sorts of analysis are compared (see below). It must be concluded therefore that the characteristic ratio for this polymer sample may be as large as $C_\infty = 14$. Until NaCMA fractions spanning a wider range of molecular weight are investigated, thereby better to define the molecular weight dependence of $[\eta]$, we incline toward the lower result for C_∞ based upon direct determination of the polymer–solvent interaction.

Discussion

Hydrodynamic data for native amylose fractions in neutral aqueous solutions reported by several workers[7,9,10] are mutually consistent in suggesting that the polymer covolume vanishes in this medium near 25°. If polydispersity of the fractions is neglected and the asymptotic value of Φ_0 is assumed, application of eq 8 to these data yields $C_\infty \simeq 5.2$. This result accords with dimensions from light scattering in the same solvents.[10,11] It is possible also to derive similar or somewhat smaller values for C_∞ from analysis of hydrodynamic data for amylose in the good solvents dimethyl sulfoxide, aqueous KOH and NaOH, and formamide.[7,9,42] The usual tendency of the extrapolation methods employed[41,43] to yield excluded volume expansions exceeding substantially the expansion expected from direct measurement of thermodynamic parameters[43,44] is evident also for amylose in good solvents in those cases for which this comparison has been made.[42,45] The procedure of Banks and Greenwood,[7,46] whereby data for the lower molecular weight samples is emphasized in making the required extrapolations, would appear to be particularly hazardous.[22,44]

Sodium carboxymethylamylose has itself been the object of a recent study by Patel, *et al.*[47] Intrinsic viscosities of NaCMA fractions, obtained by fractional precipitation and presumably possessing identical degrees of substitution, were measured in aqueous salt solutions at several ionic strengths. From the value of the Mark–Houwink K_0 observed in 0.65 M NaCl at 37.5°, which the hydrodynamic data suggest corresponds to θ conditions, one calculates $C_\infty = 7.9$, assuming the asymptotic value of Φ_0, and the reported degree of substitution. This result is vitiated by virtue of the necessary neglect of polymer heterogeneity and apparent failure to account during light scattering determinations of the molecular weights of the fractions for the multicomponent character of the system.[48] If the light scattering radii of gyration reported by Patel, *et al.*,[47] are accepted at face value, one is forced to conclude that the polymer fractions employed were very polydisperse.

Also among amylose derivatives the triacetate has been the subject of extensive study. The results of Cowie and coworkers[49,50] yield $C_\infty = 6.0$–18.0 depending upon the polymer fraction and the method of analysis. Values at the lower end of this range were invariably obtained when hydrodynamic data alone were considered;[50] the higher values resulted from light scattering radii of gyration in good solvents and appeal to thermodynamic measures of the volume expansion.[49] Patel and Patel[51] report generally higher values and an even wider range of C_∞ for the triacetate. More recent work by Greenwood and coworkers employing a variety of techniques and solvents has yielded significantly more consistent results. Light scattering radii of gyration obtained for several amylose triacetate fractions in the good solvent nitromethane,[52] corrected to ideal solvent conditions by the method of Orofino and Flory,[38] yield $C_\infty = 13.4$ and display excellent internal consistency. Using various other theoretical treatments of the polymer expansion factor to analyze the same data, virtually the same value for C_∞ resulted.[53] Sedimentation velocity experiments[54] likewise yield the similar result, $C_\infty = 12.4$. In all of these cases molecular weight heterogeneity was treated

(40) W. L. Dimpfl, to be published.
(41) W. H. Stockmayer and M. Fixman, *J. Polym. Sci., Part C*, **1**, 137 (1963).
(42) J. M. G. Cowie, *Makromol. Chem.*, **53**, 13 (1962).
(43) M. Kurata and W. H. Stockmayer, *Fortschr. Hochpolym. Forsch.*, **3**, 196 (1963).
(44) P. J. Flory, *Makromol. Chem.*, **98**, 128 (1966).
(45) J. M. G. Cowie, *ibid.*, **42**, 230 (1961).
(46) W. Banks and C. T. Greenwood, *ibid.*, **114**, 245 (1968).
(47) J. R. Patel, C. K. Patel, and R. D. Patel, *Staerke*, **19**, 330 (1967).
(48) E. F. Casassa and H. Eisenberg, *Advan. Protein Chem.*, **19**, 287 (1964).
(49) J. M. G. Cowie, *J. Polym. Sci.*, **49**, 455 (1961).
(50) J. M. G. Cowie and P. M. Toporowski, *Polymer*, **5**, 601 (1964).
(51) C. K. Patel and R. D. Patel, *Makromol. Chem.*, **90**, 262 (1966); *J. Polym. Sci., Part A-1*, **4**, 835 (1966).
(52) W. Banks, C. T. Greenwood, and D. J. Hourston, *Trans. Faraday Soc.*, **64**, 363 (1968).
(53) W. Banks and C. T. Greenwood, *Eur. Polym. J.*, **4**, 249 (1968).
(54) W. Banks, C. T. Greenwood, and D. J. Hourston, *Makromol. Chem.*, **111**, 226 (1968).

in a somewhat arbitrary fashion. Finally, intrinsic viscosities for the fractions measured in a series of mixed solvents yield $C_\infty = 9.2$ when polymer heterogeneity is ignored and $\Phi = 2.1 \times 10^{-3}$ is used.[46] Among other amylose derivatives only the tricarbanilate has been investigated with regard to its conformation in solution. Evidence for greatly increased unperturbed dimensions, relative to unsubstituted amylose, has been presented.[55]

Charged substituents on the backbone of the present NaCMA sample have an average frequency of occurrence of one for each two glucose residues. From the magnitudes of the observed second virial coefficients it is evident that mutual interaction of these charges is small in the solvent medium employed. Corrections for excluded volume expansion are consequently small as well and probably not subject to serious error despite the lack of verification of eq 11 for polyelectrolytes. *Similarity* of the backbone conformations of amylose in aqueous solution and NaCMA under the present experimental conditions is guaranteed by comparison of their iodine binding isotherms; the maximum iodine binding capacities of the two species are comparable and indicate that from 80 to 100% of the glucose residues participate in the binding interactions at saturation.[21] Random coil behavior of amylose and NaCMA in the respective solvents is evidenced by the hydrodynamic data. This may be reconciled with the necessity for a high proportion of helical structure to accommodate the bound iodine only if the helical sequences are relatively short and connected by regions of flexibility or if the helical structure is absent in the absence of iodine and is induced by its addition. (The possibility seems remote that the backbone might comprise one or a few helical sequences with sufficient integrity to bind appreciable iodine while possessing the required flexibility to display the hydrodynamic properties of a random coil.) For native amylose the negligible effect upon the intrinsic viscosity brought about by titration with iodine up to the saturation point appears to rule out the second possibility,[4] and, presumably, a high proportion of helical structure persists in aqueous solution in the absence of iodine. A similar experiment[14] with CMA solutions at pH 1.7 shows a pronounced decrease in the reduced viscosity as a function of the amount of added iodine following a narrow initial range of added iodine within which the viscosity remains constant. Thus the induction of increased helical structure in the derivative during the course of titration with iodine is indicated under some experimental conditions. These conclusions are supported by investigations of CMA–iodine binding isotherms currently underway in this laboratory.[21]

One turn of the amylose V helix, *i.e.*, the helical structure involved in complexes of amylose with numerous low molecular weight substances including iodine, is thought to be composed of from six to eight glucose units.[6] Providing that the same helical conformation is responsible for the binding of iodine to NaCMA, it is evident that the occurrence of three to four substituent groups per helix turn produces a relatively small perturbation of the structure. Nevertheless, some perturbation must be acknowledged, and the small temperature coefficient of $[\eta]$ for aqueous amylose near room temperature suggests that factors other than a difference in temperature lead to this conformational difference. In view of the small charge interaction previously noted, one must conclude that the substituent exerts a steric influence on the helix stability.

The relationship between the currently available values of C_∞ for NaCMA and amylose is consistent with the hypothesis that the proportion of helical structure in NaCMA under the present experimental conditions is less than in unsubstituted amylose in neutral aqueous media. Thus one can readily imagine conditions under which the contribution to the unperturbed chain dimensions from a helical sequence of given DP is smaller than that from a random coil sequence of the same DP. There must exist, however, a critical value for the DP above which the reverse circumstance obtains, since the helix dimensions increase as the DP whereas the unperturbed dimensions of the random coil sequence increase in the asymptote as $(DP)^{1/2}$. That this phenomenon occurs in polypeptides has been demonstrated by Nagai[56] and subsequently by Miller and Flory,[57] who used a more detailed description of the random coil sequence. These authors show that the unperturbed dimensions of polypeptides pass through a minimum as the chain is converted from helix into coil; the less cooperative the helix-to-coil transition the more pronounced will be this effect, since for any degree of helicity the population of helical sequences with DP less than the aforementioned critical value will increase as the cooperativeness is decreased. Without detailed information concerning the characteristics of helix and coil states for amylose one cannot specify the critical DP at which helix and coil sequences contribute equally to the unperturbed chain dimensions. Preliminary calculations[40] following the method of Miller and Flory[57] and using reasonable models (based on conformational energy calculations[40]) for sequences capable of binding iodine (V helix) and sequences incapable of iodine binding ("coil") show clearly, however, that conversion of amylose helix to coil may result in an increase in the unperturbed chain dimensions throughout most of the range of helix content, *i.e.*, for helix content below *ca.* 90%. Low degrees of cooperativeness were required, as expected, in order to match the observed coil dimensions of amylose at high degrees of helicity. These ideas regarding the conformation of NaCMA are currently under further investigation; unperturbed dimensions of CMA and other amylose derivatives are being studied as a function of solvent medium, degree of substitution, and charge density, and theoretical interpretation is being attempted. The results will be published in due course.

Finally, the relationship of C_∞ for NaCMA and amylose triacetate is noteworthy. Examination of space-filling molecular models reveals the limited range of conformation space available to a maltose unit of

(55) W. Burchard, *Z. Phys. Chem.* (Frankfurt am Main), **42**, 293 (1964); *Makromol. Chem.*, **88**, 11 (1965).

(56) K. Nagai, *J. Chem. Phys.*, **34**, 887 (1961).

(57) W. G. Miller and P. J. Flory, *J. Mol. Biol.*, **15**, 298 (1966).

amylose, and this finding is verified by conformational energy calculations.[33,40] Molecular models for maltose triacetate reveal an almost identical range of conformation space free of serious steric conflicts. To the extent that only those interactions present in the maltose moiety are responsible for determining the unperturbed chain dimensions, amylose triacetate should resemble amylose or carboxymethylamylose. To the extent that conformations involving longer range interactions, such as occur in helical portions of amylose, make important contributions to the average chain properties, amylose triacetate will not resemble amylose. The probable crystalline helical structure of amylose triacetate involves fewer than five glucose units per turn,[34] but there is no evidence for retention of this conformation in dilute solution. The observation that C_∞ for NaCMA falls between those for amylose and amylose triacetate is thus consistent with the model proposed for NaCMA which involves a moderate disruption of the helical sequences of native amylose.

Theory of Long-Chain Molecules with Partial Flexibility

Yukiko Tagami[1]

Mellon Institute, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213.
Received March 21, 1968

ABSTRACT: The conformation of a linear, semiflexible, long-chain molecule in a Θ solvent is described in terms of the theory of Markoffian processes due to Uhlenbeck and Ornstein. The model exhibits correct behavior in both the limiting cases of a completely flexible chain and a thin rigid rod, and gives a fair approximation to the wormlike chain at intermediate degrees of stiffness. In contrast to the wormlike chain, the present model permits analytical evaluation of all the higher moments of the end-to-end distance and thus offers a useful treatment of the angular distribution function for intensity of Rayleigh scattering.

I. Introduction

The conformation of linear, incompletely flexible, long-chain molecules in dilute solution in a Θ solvent is usually characterized by the spatial correlation between different points on the chain. The approach of the conformation of a long-chain molecule, in the limit of a sufficiently long chain, to that of an assembly of normally distributed point masses is well established. Moreover, when chain flexibility is introduced as a parameter, the resulting non-Gaussian conformation can be described in terms of the well-known "wormlike" chain[2-5] or a related model.[6,7]

The conformation of a polymer chain is conveniently represented, as in Figure 1, in terms of the set of position vectors, $\mathbf{r}(s)$, drawn from an arbitrary origin to points along the chain contour designated by a parameter $0 \leq s \leq L$ running from one end of the chain of length L consisting of n segments. In the wormlike chain models, the statistical fluctuations in length of a chain $\mathbf{r}(s) - \mathbf{r}_0$, with $\mathbf{r}_0$ designating $\mathbf{r}(0)$, are assumed to be attributable entirely to a slight bending of the chain. In this paper we consider a different correlation scheme, in which, instead of seeking the physical mechanism for the fluctuations in length, we merely assume the resultant fluctuations to be random. The outcome is that our correlation function introduces a dependence of $\mathbf{r}(s) - \mathbf{r}_0$ on the most probable value $\langle \mathbf{r}(s) - \mathbf{r}_0 \rangle_{\mathbf{u}_0}$, with the orientation of the first chain segment vector $\mathbf{u}_0 = (\partial \mathbf{r}/\partial s)_{s=0}$ specified.

In sections II, III, and IV, we will develop the statistical picture of our model in a derivation based on the diffusion process described by Uhlenbeck and Ornstein.[8,9] The validity, and the limitations, of the "O–U process," as it is often called, are discussed for our application. In section V, our model is compared with others. It is shown that while the features of all these models are contained in our model as specific limiting cases, independent assumptions made in our derivation give rise to some new aspects not possessed by the previous models.

The present work was inspired by the fact that the diffusion process discussed by Uhlenbeck and Ornstein obviates a difficulty encountered in the description of ordinary Brownian motion wherein the particle velocity appears to approach infinity as the time lapse becomes very short. The importance of this property of the model will be made apparent by consideration

(1) Address correspondence to the author at the Department of Chemistry, Stanford University, Stanford, California 94305.

(2) O. Kratky and G. Porod, *Rec. Trav. Chim. Pays-Bas*, **68**, 1106 (1949).

(3) J. J. Hermans and R. Ullman, *Physica*, **18**, 951 (1952).

(4) H. E. Daniels, *Proc. Roy. Soc.* (Edinburgh), **A63**, 290 (1952).

(5) A. Peterlin in "Electromagnetic Scattering," M. Kerker, Ed., Pergamon Press, London, 1963, p 357.

(6) R. A. Harris and J. E. Hearst, *J. Chem. Phys.*, **44**, 2595 (1966).

(7) J. E. Hearst and R. A. Harris, *ibid.*, **46**, 398 (1967).

(8) G. E. Uhlenbeck and L. S. Ornstein, *Phys. Rev.*, **36**, 823 (1930) (reprinted in "Selected Papers on Noise and Stochastic Processes," N. Wax, Ed., Dover Publications, New York, N. Y. 1954).

(9) S. Chandrasekhar, *Rev. Mod. Phys.*, **15**, 1 (1943); also in "Selected Papers on Noise and Stochastic Proccesses," N. Wax, Ed., Dover Publications, New York, N. Y., 1954). This paper contains an extensive list of references relevant to the derivation of the O–U process.