Conformational Transition of Hyaluronic Acid in Aqueous–Organic Solvent Monitored by Vacuum Ultraviolet Circular Dichroism†

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ABSTRACT: The chiroptical transition of hyaluronic acid (HA) in aqueous–organic solvent has been investigated by circular dichroism (CD) spectroscopy into the vacuum ultraviolet region. The CD of HA changes dramatically, monitoring a cooperative transition as the dielectric constant of an aqueous solution is reduced by adding organic solvents. This transition results in a high-intensity CD band at 188 nm, indicating an ordered structure in the mixed solvent. Heating HA in the mixed solvent also causes a cooperative transition, reducing the CD to that found for the polymer in aqueous solution. In contrast, heating HA in aqueous solution results in small, noncooperative changes in the CD spectrum. This indicates an unordered structure in aqueous solution. The CD as the dielectric constant is reduced exhibits isodichroic points, showing that there are only two environments for chromophores contributing to the CD. This is confirmed by singular value decomposition of CD spectra recorded as a function of solvent composition, which shows the spectra to contain only two principal components. The data describing the thermally induced transition of HA in mixed solvent are not consistent with infinite cooperativity. The van't Hoff relation yields thermodynamic parameters for the conformational transition in terms of the cooperative unit of −60 kcal mol^{-1} for $\Delta H°$ and −180 eu mol^{-1} for $\Delta S°$.

Hyaluronic acid (HA)[1] is a copolymer of 2-*N*-acetyl-2-deoxy-D-glucopyranose residues in β-1,4 linkage strictly alternating with D-glucopyranoside uronic acid residues in β-1,3 linkage (Meyer, 1958). As the simplest glycosaminoglycan (GAG), it does not contain any sulfate groups that create microheterogeneity and increase linear charge density in other GAGs. Several physiological functions have been proposed for HA, such as that of a shock absorber and lubricant in articular cartilage (Preston et al., 1965), a filter regulating flow in the vitreous of the eye (Balazs & Gibbs, 1970), and a modifier of cell behavior during differentiation (Derby, 1978) and tumor invasion (Toole et al., 1979). The molecule has also been suggested to serve as a mechanoelectrical transducer in the inner ear (Barrett, 1975, 1976; Barrett & Harrington, 1977), an effector of tissue deformability during pregnancy (Golichowski et al., 1980), a maintainer of passageways for cellular migrations during development (Fisher & Solursh, 1977), an enhancer of diffusion of small molecules (Napier & Hadler, 1978), and an organizer of proteoglycan molecules in cartilage (Hascall, 1977).

Most of the functions proposed for HA have some basis in the expansive nature of the molecule, which can occupy a domain 10^3–10^4 times the volume of the polymer chain itself (Balazs, 1958). The polymer has been characterized in solution as a random coil with some stiffness (Laurent, 1970; Preston et al., 1965). In investigation of the source of high viscosity for HA in solution, considerable evidence for conformational order has accumulated. This includes the results of studies based on viscometry (Swann & Caulfield, 1975), nuclear magnetic resonance (Darke et al., 1975), periodate oxidation kinetics (Scott & Tigwell, 1978), rheology (Morris et al., 1980a,b; Welsh et al., 1980), and optical activity (Chakrabarti & Balazs, 1973; Cowman et al., 1981, 1983; Park & Chakrabarti, 1978a,b).

Stone (1964, 1965) pioneered chiroptical studies by investigating optical rotatory dispersion induced in the transitions of symmetric dyes upon binding to various GAGs. Later, CD measurements revealed a correlation between the sign of the band in the energy region common to π–π^* transitions and the amino sugar linkage (Stone, 1969, 1971). The CD spectrum of HA polymer has been shown to differ either from a simple algebraic sum of its monomer spectra (Buffington et al., 1977) or from spectra of its tetra- or hexasaccharides (Chakrabarti & Balazs, 1973). Cowman et al. (1981, 1983) have demonstrated the enhanced CD of HA polymer in aqueous solution to arise from a local effect, which does not suggest any long-range conformational order in the polymer.

Park and Chakrabarti (1977, 1978a,b) observed a transition in the near-ultraviolet CD spectrum of HA upon lowering the pH and raising the fraction of organic component in a mixed solvent. They indicated that the transition is reversed upon raising the pH or temperature of the sample and is cooperative in nature. The transition was not observed either in chemically degraded HA or in its isomer, chondroitin. Furthermore, the chiroptical transition could be correlated with viscosity changes in the HA solution (Park & Chakrabarti, 1978b). The CD spectrum of HA in aqueous–organic solution at low pH resembles the spectrum of a HA film (Buffington et al., 1977). It was therefore suggested to represent HA in one of the 4-fold helical conformations proposed from X-ray diffraction studies (Guss et al., 1975).

We have measured a CD spectrum for carefully deionized HA at low pH both in aqueous solution and in mixed aqueous–organic solvent. Our spectra are extended into the vacuum ultraviolet region to wavelengths shorter than 180 nm, clearly showing the intense negative CD of HA in the mixed solvent

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[1] Abbreviations: GAG, glycosaminoglycan; CD, circular dichroism; HA, hyaluronic acid; NaHA, hyaluronic acid sodium salt; NaGlcUA, D-glucuronic acid sodium salt; GlcNAc, *N*-acetyl-D-glucosamine; SVD, singular value decomposition; OD, optical density; LD, linear dichroism.

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to be centered at 188 nm. We extend normal absorption spectra into the vacuum UV to confirm that this band is due to a $\pi-\pi^*$ transition in the acetamido group of HA. We monitor the effect of solvent composition, temperature, and pH on the CD of HA in the $\pi-\pi^*$ transition region. In general, our results agree with the conclusions of Park and Chakrabarti (1978a,b) reached by measuring CD spectra of the $n-\pi^*$ transition in the near-ultraviolet region. However, there are important differences between our results and those of the earlier workers which are likely due to experimental factors such as high order sample aggregation and instrumental limitations.

We present CD spectra of HA at low pH recorded as a function of solvent composition and show how SVD (Forsythe et al., 1977) can be used to create a basis set of orthogonal elements. There are only two significant elements in this basis set, demonstrating that the chromophores of HA responsible for the changes in CD observed as a function of solvent composition have only two allowable environments. The transition in a mixed aqueous–organic solvent can be reversed by raising the temperature, and we use the CD for the melting of the mixed-solvent HA structure to determine the thermodynamic parameters for the transition.

Experimental Procedures

Sample Preparation. Monovalent salts of HA polymer were purchased from Sigma Chemical Co. (bovine vitreous humor, grade IV) and Calbiochem-Behring. Material molecular weights (M_r) were estimated from intrinsic viscosity measurements in 0.2 M NaCl solution at 25 °C, using the relation $[\eta] = 0.228M_r^{0.816}$ (Cleland & Wang, 1970). Viscosity measurements were performed in a semimicro version of the floating rotor couette viscometer of Zimm and Crothers (1962). The molecular weight of the Calbiochem HA was determined to be 170000, and that of the Sigma material was 240000.

Unless stated otherwise, mixed solvent refers to a solution containing ethanol at a concentration of 45% by volume to volume of solution, or 7.92 M. Acidic mixed solvent contains phosphoric acid and has an aqueous component with a pH of 2.5. HA polymer was prepared for spectroscopy by deionizing an aqueous solution with a strong cation-exchange resin in the proton form (AG 50W, Bio-Rad), followed by lyophilization and solvation in water. Solutions of polymer sample were typically unbuffered, using phosphoric acid to lower the pH and containing one of several different nonpolar solvents at various concentrations. We used ethanol, 2,2,2-trifluoroethanol, and acetonitrile as nonpolar solvents, all three of which produced very similar spectral effects on HA. The nonpolar solvents were gravimetrically added to an acidic aqueous solution of HA, with further addition of water to known final volume after mixing. Alternatively, aqueous solutions of polymer were dialyzed 4 times against a 500-fold excess of the desired solvent. All water used in these experiments was glass distilled.

Spectroscopy. HA concentrations were based on the colorimetric determination of glucuronic acid (Bitter & Muir, 1962) and are given per mole of the fundamental NaGlcUA–GlcNAc disaccharide unit. For spectroscopic assay of concentration, absorption measurements were performed on a Cary 15 or Cary 219 spectrometer with nitrogen purge. The results were compared with colorimetric determinations of HA concentration to establish extinction coefficients for the polymer at 190 nm under various solution conditions.

Circular dichroism spectra were recorded by using a vacuum ultraviolet spectrograph described previously (Johnson, 1971). The CD signal was calibrated with (+)-10-camphorsulfonic acid (Aldrich) in a 1-mm cell, assuming a $\Delta\epsilon$ of 2.41 M^{-1} cm^{-1} at 290.5 nm. Most commonly, a spectral bandwidth of 1.6 nm and a scan rate of 0.5 nm/min were used. Samples were contained in cylindrical cells (Hellma) with nominal path lengths of 0.01, 0.05, 0.1, 0.2, and 1.0 mm. For all CD measurements, the total optical density of sample, solvent, and cell was less than 1.0. Path lengths of cells 0.1 mm and shorter were determined interferometrically (Bree & Lyons, 1956). Longer path lengths were checked by absorption measurements of a sample of known concentration and extinction coefficient. Spectra recorded with a thin-cell apparatus, in which the sample is sandwiched between two quartz windows with an effective path length of 2–20 μm, were scaled to provide agreement with results from longer path-length cells in the spectral region common to the two measurements.

The temperature of the sample cell was controlled by a brass jacket through which glycol–water of constant temperature was cycled by a Lauda recirculating bath. A thermistor probe (Yellow Springs Instruments) at the edge of the cell face was calibrated to measure accurate temperatures for the sample.

Data Analysis. Series of CD spectra recorded as a function of temperature or solvent composition were analyzed for orthogonal components (Hennessey & Johnson, 1981) by using a matrix decomposition technique called singular value decomposition (Forsythe et al., 1977). Approximations of the original data spectra were created by using only the significant components of the basis set, thus filtering noise from the spectral data, while indicating the information content of the data set.

Results and Discussion

Monomer Spectra. CD spectra of the HA monomers are presented here primarily as a reference point for comparison with the polymer spectra. Those spectral features of polymeric HA not found in the monomer spectra may be important in reflecting conformational order and the existence of chromophore environments not possible for the monomers. Care must be exercised in interpreting such differences since they may arise from other sources as well, such as the formation of new covalent features (glycosidic bonds) and chromophores (acetal from hemiacetal) which should influence the interactions among parts of the molecule that produce optical activity. We will see that the CD changes observed in polymer spectra upon altering the dielectric constant of the solvent are far more dramatic than the corresponding changes in the monomer spectra.

The CD spectrum of GlcUA measured in aqueous solution at pH 2.5 (Figure 1, top panel) is in agreement with the results of Buffington et al. (1977) for wavelengths longer than 190 nm. We have extended the solution spectrum further into the ultraviolet and observe a negative band centered at 183 nm with a $\Delta\epsilon$ of -1.42 M^{-1} cm^{-1}, corresponding in position and sign to the band observed by Buffington et al. (1977) in GlcUA film. The longer wavelength bands of opposing sign are believed to arise from $n-\pi^*$ transitions in different rotational isomers of the uronic acid (Listowsky et al., 1969). The 183-nm band has been interpreted as due to a carboxyl $\pi-\pi^*$ transition in the molecule (Buffington et al., 1977). Introduction of ethanol at 45% v/v (Figure 1, top) induces a slight blue shift of the CD crossover at 225 nm and a modest decrease in CD throughout the spectrum. A change in temperature from 24 to 50 °C has little effect on the GlcUA spectrum in aqueous or mixed solvent.

The negative CD band at 210 nm for GlcNAc in aqueous solution (Figure 1, bottom panel) has been assigned to an $n-\pi^*$

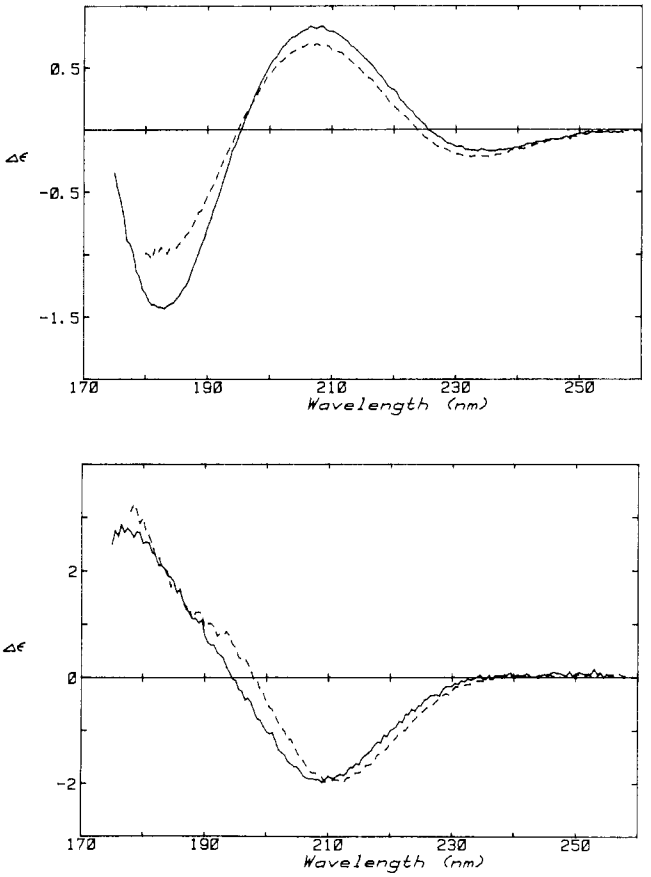

FIGURE 1: CD spectra of HA monomers. (Top) CD spectra of GlcUA recorded in an aqueous solution 12.5 mM in NaH_2PO_4 and 7.5 mM in H_3PO_4, pH 2.5, in the absence (—) and in the presence (---) of 45% v/v ethanol (7.92 M). Spectra were recorded at 24 °C in 52.4- and 11.9-μm cylindrical cells at 51 mM concentration of GlcUA. (Bottom) CD spectra of GlcNAc in the same solution condition as above, in the absence (—) and in the presence (---) of 45% v/v ethanol. GlcNAc concentration was approximately 15 mM.

transition in the acetamido chromophore (Buffington et al., 1977). It is a common feature in the CD spectra of glycosaminoglycans, as originally reported by Stone (1969). Our value for $\Delta\epsilon_{210}$ is somewhat larger than that previously reported (Buffington et al., 1977; Cowman et al., 1983), and we have traced this to the lower pH of our solvent system. This CD spectrum displays no clear band maximum in the π–π^* transition region. Addition of ethanol to the solution does result in the appearance of a positive shoulder in this region, which coincides with a maximum in the normal absorption spectrum of the molecule at 187 nm ($\epsilon_{187} \sim 9000$ M^{-1} cm^{-1}). The strong absorption is consistent with the assignment of CD in this region to a π–π^* transition, by Buffington et al. (1977).

Polymer CD in Aqueous Solution. The CD spectrum of HA polymer in aqueous solution at pH 2.5 (Figure 2) consists of negative CD in the n–π^* transition region near 210 nm and an approach to the base line in the π–π^* region near 190 nm, with negative CD at shorter wavelengths. Thin-cell measurements at room temperature show that the second negative CD band in HA is centered at a wavelength shorter than 173 nm and is at least 1.5 times the intensity of the 210-nm band. Surprisingly, there is little CD in the π–π^* region considering that both the acetamido and the carboxyl groups of HA should have transitions there. Buffington et al. (1977) proposed that cancellation of monomer transitions resulted in the weak signal of HA at 190 nm. In comparing monomer and polymer spectra, they were able to show that the changes in HA CD resulting from an increase in pH are paralleled by similar

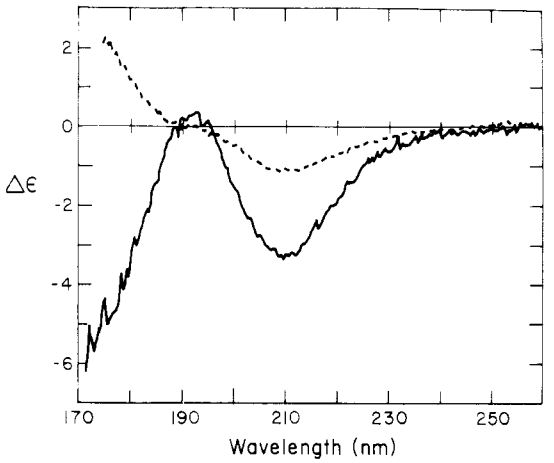

FIGURE 2: CD spectrum of HA per disaccharide recorded in aqueous solution buffered at pH 2.5 with 20 mM sodium phosphate (—). For comparison, a spectrum which is the sum of the component monomer spectra is also presented (---).

changes in the CD of GlcUA.

We have observed subtle changes as a function of pH in absorption and flow LD spectra for the π–π^* transition region of HA. At pH 6.8, the flow LD spectrum contains a broad and negative band from 175 to 190 nm which appears to contain two transitions. With acidification, the total signal intensity decreases, and the shape of the LD signal also changes, indicating the presence of distinct transitions at wavelengths of 187 nm and shorter than 180 nm. These results, when combined with the presence of absorption maxima for HA and GlcNAc at 187 nm and the absence of such a maximum in the corresponding spectrum of GlcUA, suggest that the π–π^* transition of GlcNAc in HA lies near 187 nm, with the π–π^* transition of GlcUA occurring at a shorter wavelength.

For comparison with the CD spectrum of HA, a sum of monomer spectra has been included (Figure 2). The polymer has far more negative CD than the monomer sum in the n–π^* transition region. At wavelengths shorter than 190 nm, the two spectra differ in sign. The difference between the HA polymer and the combined monomer spectra has been noted previously, and various origins for it have been discussed, including conformational order within the polymer backbone (Chakrabarti & Balazs, 1973), changes in average chromophore orientations in monomers as affected by neighboring residues (Buffington et al., 1977), and the conversion of hemiacetal chromophores in the monosaccharides to acetal chromophores with glycosidic bond formation in the polymer (Cowman et al., 1983).

As HA polymer in aqueous solution at pH 2.5 is heated (Figure 3), the decrease in CD magnitude of the 210- and 190-nm bands is approximately linear with temperature in the range of 0–60 °C. There is an apparent isodichroic point at $\Delta\epsilon_{197} = -0.5$ M^{-1} cm^{-1}, and analysis by SVD confirms that the spectra are composed of varying proportions of only two basis spectra. Further characterization of this CD change is beyond the scope of the present work. Its nature suggests that it reflects a change in HA structure involving a small energy, and in which it is unlikely that many residues cooperate.

Polymer CD in Mixed Solvent. Changes in CD that occur with introduction of an organic solvent into aqueous HA solutions can be quite striking and are cooperative in nature. With addition of ethanol (Figure 4), the appearance of strong negative CD at 187 nm is accompanied by loss of negative signal in the n–π^* transition region of 210 nm. The change is essentially complete at 25% ethanol, and this spectrum is

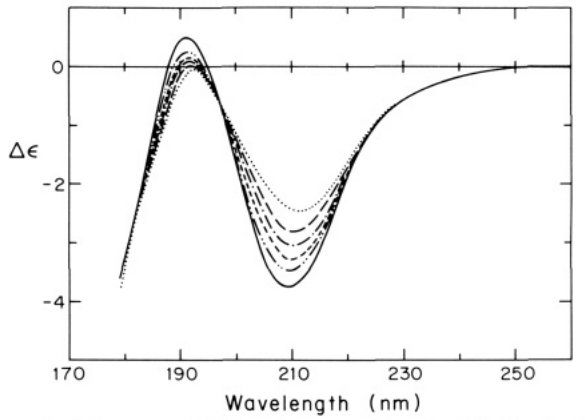

FIGURE 3: CD spectra of HA polymer per disaccharide dissolved in aqueous solution, pH 2.5, recorded as a function of temperature. Temperatures in degrees centigrade were the following: −1 (—), 12 (−··−), 20 (- - -), 32 (−·−), 47 (−−), 62 (···). For clarity in presentation, these data were smoothed by drawing a line through the noise.

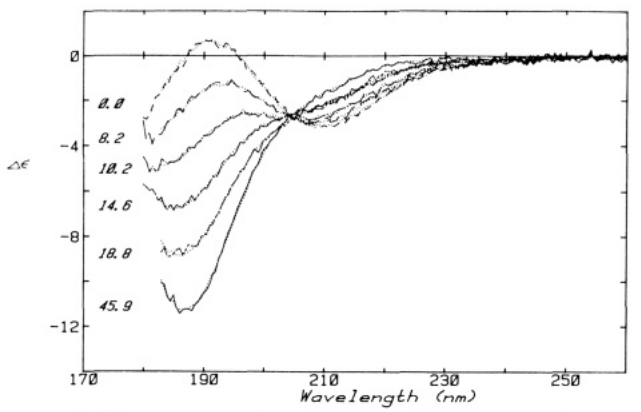

FIGURE 4: CD spectra per disaccharide at 25 °C for HA dissolved in solutions with various proportions of ethanol to aqueous phosphoric acid solution, pH 2.5. Dotted curves are reconstructions of the sample spectra using only the two most significant components of an orthogonal basis set derived from these spectra and 10 others (Figure 5). The reconstructed spectra contain all of the CD information greater than the noise level of the measurements.

similar to that observed for NaHA films (Buffington et al., 1977) with a blue shift of the strong negative CD band from 194 nm in film to 187 nm in the acidic mixed solvent. Measurements of solutions with 2,2,2-trifluoroethanol substituted for ethanol reveal an isodichroic point near $\Delta\epsilon_{176} = -5.0\ M^{-1}\ cm^{-1}$ in addition to the one at $\Delta\epsilon_{204.5} = -2.7\ M^{-1}\ cm^{-1}$. Measurements to 169 nm using a thin-cell apparatus show a crossing of the base line near 175 nm, with positive CD at shorter wavelengths. The presence of an isodichroic point demonstrates that the spectral series is composed of linear combinations of only two distinct species. We confirm this and simultaneously filter noise from the spectra by using SVD (Figure 5). This does not necessarily mean that any conformational transition of the polymer molecule as a whole, reflected in this CD change, is a two-state one. It does imply that those chromophores in HA contributing to the CD change with solvent experience one of only two environments.

Park and Chakrabarti (1977) were the first to measure the CD of a chiroptical transition of HA as a function of solvent composition. They indicated cooperativity and reversal of the transition with increase in pH or temperature, but no systematic series of spectra was presented. They also correlated the change in CD with a change in intrinsic viscosity of the polymer solution (Park & Chakrabarti, 1978b). These authors utilized the wavelength region near 225 nm to describe this

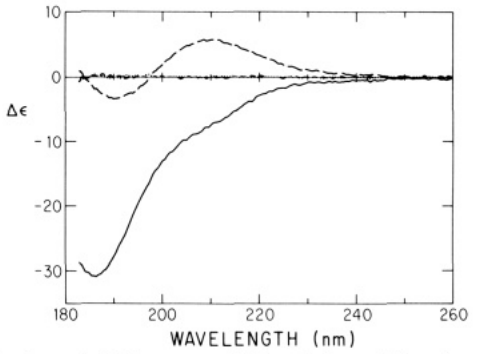

FIGURE 5: A total of 16 spectra of HA solutions with various alcohol contents including those of Figure 4 were analyzed for orthogonal components using SVD (Hennessey & Johnson, 1981; Forsythe et al., 1977). The four most significant components are presented. All of the useful spectral information is contained in the two components with the largest eigenvalues. Eigenvalues for basis curves are 23 980 (—), 1134 (−−), 4.36 (- - -), and 3.31 (···).

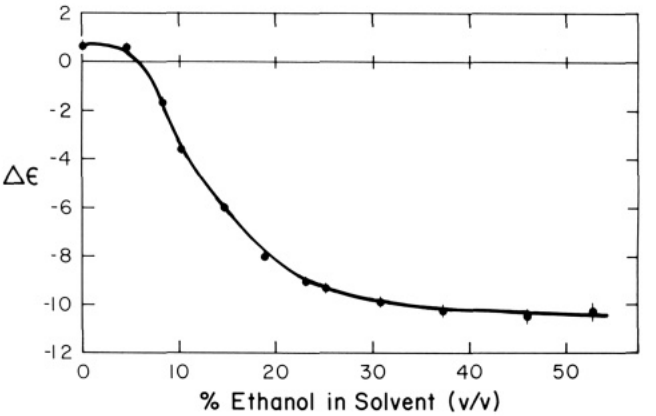

FIGURE 6: CD transition curve at 190 nm for HA as a function of ethanol concentration.

chiroptical transition, but the shorter wavelengths studied here are much more sensitive (Figure 6). The midpoint for the cooperative change with increasing ethanol content of the solvent as monitored by $\Delta\epsilon_{190}$ is approximately 12.5% v/v ethanol at 24 °C and 2–3 mM concentration of disaccharide. The transition is nearly complete at 25% v/v ethanol. These results are in agreement with those of Park and Chakrabarti obtained by monitoring the chiroptical transition at 225 nm. However, we do not observe positive CD in the 225-nm region as presented by Park and Chakrabarti (1978a,b), either for oligomers of HA in buffered mixed solvent as discussed in the accompanying paper (Staskus & Johnson, 1988) or for deionized polymer in acidic mixed solvent shown here. We have observed positive CD signals in spectra of HA polymer, but only in the presence of monovalent counterion, which at low pH induces polymer gel formation at the concentrations used in our experiments. Such spectral results are not reliable due to LD contributions to the CD. We suggest that the positive CD at 225 nm observed by Park and Chakrabarti results either from sample orientation, becoming more pronounced with large-scale polymer aggregation into networks in the presence of counterion, or from differential scattering of the circularly polarized light by such aggregated material (Bustamante et al., 1983). Furthermore, Park and Chakrabarti (1978a) describe a concentration dependence of the positive CD at 225 nm, whereas we observe no such dependence in our spectra of deionized HA polymer. If the positive CD at 225 nm is a result of large-scale aggregation of sample material, it would be expected to display sensitivity to sample concentration.

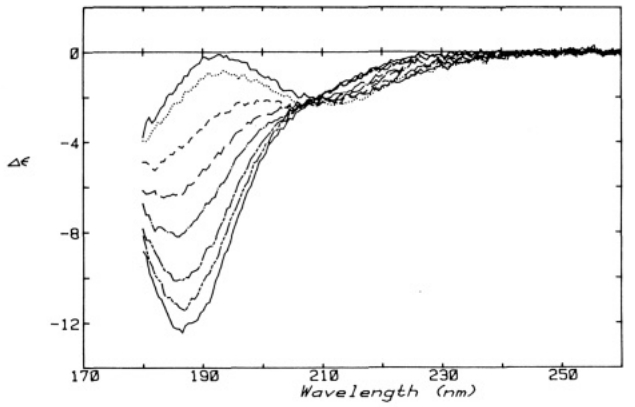

FIGURE 7: CD spectra of HA per disaccharide recorded at various temperatures in an acidic aqueous solution containing 6.34 M acetonitrile. Temperatures in degrees centigrade were as follows: 2.3 (—), 22.3 (– – –), 30 (– –), 35 (– · –), 38 (– –), 40 (– – –), 45 (···), 64 (—). The CD spectrum at 15 °C is only slightly different from the spectrum at 2.3 °C.

Park and Chakrabarti do not present data for HA solutions with ethanol concentrations greater than ~25% v/v. Using deionized HA polymer, we find that we can increase the ethanol content of our solutions in excess of 50% v/v without large-scale aggregation of the HA. In the process, we observe small CD changes when the alcohol content is increased beyond 25% v/v. In subsequent work, we utilize an ethanol concentration of 45% v/v, as it appears to represent a true end point in the CD changes observed as a function of ethanol concentration.

We have made several attempts to measure the CD spectrum of unoriented polymer gel in 45% v/v ethanolic solvent, pH 3, containing 20 mM sodium ion. These attempts included heating the sample to melt the gel while introducing it into the CD cell, squashing the gel material between quartz windows in a pseudorandom fashion, and adding an aliquot of concentrated buffer solution to deionized polymer in acidic mixed solvent within a cell. This last approach was the most effective for achieving a CD spectrum of a sample with minimal orientation as judged by LD. The CD of such samples at 187 nm was approximately -15 M^{-1} cm^{-1} at 25 °C, which is still perhaps an overestimate of the magnitude. This value is considerably smaller in magnitude than the estimate of -21 M^{-1} cm^{-1} at 189 nm by Park and Chakrabarti (1978a) for deionized HA in 20% ethanol solution at pH 3.

Temperature Dependence of the CD of HA in Mixed Solvent. Acetonitrile produces the same spectral effect on HA as ethanol and was utilized for temperature studies because it eliminates concern that the sample may be esterified. The changes in HA CD observed with increasing temperature in acidic mixed solvent (Figure 7) are essentially a reversal of those resulting from addition of ethanol (or acetonitrile) to an acidic aqueous solution of HA (Figure 4). With increasing temperature, the negative CD in the π–π^* transition region of 187 nm is lost, with a concomitant increase of negative CD in the n–π^* transition region at 210 nm.

Unlike the situation with varying solvent composition, there is no clear isodichroic point for the CD as a function of temperature. Reconstructions of the original spectra within the noise level of the measurements requires a small amount of a third element from the SVD basis set. There are still essentially two environments for the chromophore, but the CD of HA in acidic aqueous solution, which is representive of the CD for unordered HA in both solvents, is observed to change with temperature in a noncooperative manner (Figure 3). Above 60 °C, the CD spectrum of HA in acidic mixed solvent

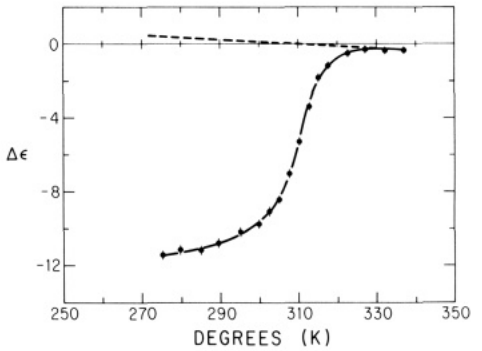

FIGURE 8: CD melting curve at 190 nm for HA in water–acetonitrile solution (—). The wavelength was chosen for its proximity to the negative CD maximum at low temperature (187 nm) and the low intensity of CD observed at 190 nm in aqueous HA solutions (– – –). Vertical bars represent the noise level in the measurements.

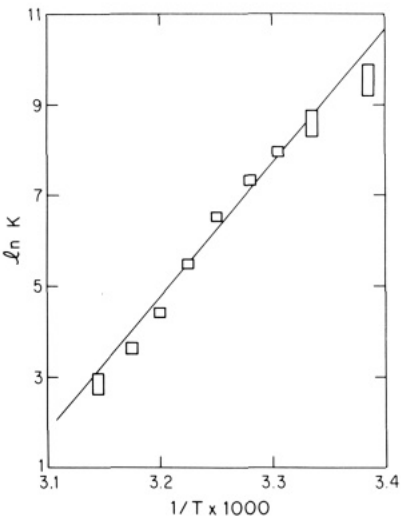

FIGURE 9: van't Hoff plot of data of Figure 8. $\Delta\epsilon_{190}$ for the low-temperature limit of the transition was assumed to be -11.8 M^{-1} cm^{-1} (independent of temperature), and the high-temperature limit was assumed to be the same as that in acidic aqueous solution. Boxes show repeatability from various measurements.

(Figure 7) is nearly identical with its spectrum in aqueous solution at that temperature (Figure 3). This identity supports our supposition that HA is essentially unordered in aqueous solution and that introduction of organic solvent has little effect on the CD of unordered HA. The small differences between these spectra in band amplitudes and frequencies are similar in nature to those spectral changes observed in the monomer CD upon introduction of organic solvent.

The $\Delta\epsilon_{190}$ for HA in mixed solvent as a function of temperature (Figure 8) can be analyzed in terms of a simple model for two chromophore environments. Briefly, the CD at 190 nm can be used to estimate the fractions of ordered and unordered chromophore at various temperatures. From these values, an equilibrium constant K for the transition between ordered and unordered chromophores can be calculated by using one of various models involving an intra- or interstrand reaction. A plot of the logarithm of K versus $1/T$, where T is the absolute temperature, will provide an estimate of the transition enthalpy from the slope of the graph following the van't Hoff relation. The van't Hoff plot (Figure 9) is nearly linear. The slight nonlinearity may be due to temperature dependence of the transition enthalpy, failure of the simple model to completely describe the transition, or both.

We use the van't Hoff relation with assumptions that (1) this is a two-strand association, (2) the CD of unordered HA in acidic mixed solvent is the same as that of unordered HA

in acidic aqueous solution at the same temperature, and (3) the minimum $\Delta\epsilon_{190}$ for ordered HA is -12 M^{-1} cm^{-1} (a pragmatic compromise among the values of -11, -12, and -15 M^{-1} cm^{-1} which gives a good fit). We obtain a $\Delta H°$ of -60 kcal mol^{-1} and a $\Delta S°$ of -180 eu mol^{-1}. If the transition were noncooperative, these units would be per mole of chromophore. If the transition were infinitely cooperative (two-state), the units would be per mole of polymer. Here, we have finite cooperativity, and the units are per mole of an intermediate length "cooperative unit". The large values of $\Delta H°$ and $\Delta S°$ show that the transition is quite cooperative. This leads us to treat the transition more rigorously in the following paper (Staskus & Johnson, 1988) by using oligomers.

Registry No. Hyaluronic acid, 9004-61-9.

REFERENCES

Balazs, E. A. (1958) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 17*, 1086–1093.

Balazs, E. A., & Gibbs, D. A. (1970) in *Chemistry and Molecular Biology of the Intercellular Matrix* (Balazs, E. A., Ed.) Vol. 3, pp 1241–1253, Academic, New York.

Barrett, T. W. (1975) *Biochim. Biophys. Acta 385*, 157–161.

Barrett, T. W. (1976) *BioSystems 8*, 103–109.

Barrett, T. W., & Harrington, R. E. (1977) *Biopolymers 16*, 2167–2188.

Bitter, T., & Muir, H. M. (1962) *Anal. Biochem. 4*, 330–334.

Bree, A., & Lyons, L. E. (1956) *J. Chem. Soc.*, 2658–2670.

Buffington, L. A., Pysh, E. S., Chakrabarti, B., & Balazs, E. A. (1977) *J. Am. Chem. Soc. 99*, 1730–1734.

Bustamante, C., Tinoco, I., Jr., & Maestre, M. F. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 3568–3572.

Chakrabarti, B., & Balazs, E. A. (1973) *J. Mol. Biol. 78*, 135–141.

Cleland, R. L., & Wang, J. L. (1970) *Biopolymers 9*, 799–810.

Cowman, M. K., Balazs, E. A., Bergmann, C. W., & Meyer, K. (1981) *Biochemistry 20*, 1379–1385.

Cowman, M. K., Bush, C. A., & Balazs, E. A. (1983) *Biopolymers 22*, 1319–1334.

Darke, A., Finer, E. G., Moorhouse, R., & Rees, D. A. (1975) *J. Mol. Biol. 99*, 477–486.

Derby, M. A. (1978) *Dev. Biol. 66*, 321–336.

Fisher, M., & Solursh, M. (1977) *J. Embryol. Exp. Morphol. 42*, 195–207.

Forsythe, G. E., Malcolm, M. A., & Moler, G. B. (1977) in *Computer Methods for Mathematical Computations*, Prentice-Hall, Englewood Cliffs, NJ.

Golichowski, A. M., King, S. R., & Mascaro, K. (1980) *Biochem. J. 192*, 1–8.

Guss, J. M., Hukins, D. W. L., Smith, P. J. C., Winter, W. T., Arnott, S., Moorhouse, R., & Rees, D. A. (1975) *J. Mol. Biol. 95*, 359–384.

Hascall, V. C. (1977) *J. Supramol. Struct. 7*, 101–120.

Hennessey, J. P., Jr., & Johnson, W. C., Jr. (1981) *Biochemistry 20*, 1085–1094.

Johnson, W. C., Jr. (1971) *Rev. Sci. Instrum. 42*, 1283–1286.

Laurent, T. C. (1970) in *Chemistry and Molecular Biology of the Intercellular Matrix* (Balazs, E. A., Ed.) Vol. 2, pp 703–732, Academic, New York.

Listowsky, I., Englard, S., & Avigad, G. (1969) *Biochemistry 8*, 1781–1785.

Meyer, K. (1958) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 17*, 1075–1077.

Morris, E. R., Rees, D. A., Robinson, G., & Young, G. A. (1980a) *J. Mol. Biol. 138*, 363–374.

Morris, E. R., Rees, D. A., & Welsh, E. J. (1980b) *J. Mol. Biol. 138*, 383–400.

Napier, M. A., & Hadler, N. M. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 2261–2265.

Park, J. W., & Chakrabarti, B. (1977) *Biopolymers 16*, 2807–2809.

Park, J. W., & Chakrabarti, B. (1978a) *Biopolymers 17*, 1323–1333.

Park, J. W., & Chakrabarti, B. (1978b) *Biochim. Biophys. Acta 544*, 667–675.

Preston, B. N., Davies, M., & Ogston, A. G. (1965) *Biochem. J. 96*, 449–474.

Scott, J. E., & Tigwell, M. J. (1978) *Biochem. J. 173*, 103–114.

Staskus, P. W., & Johnson, W. C., Jr. (1988) *Biochemistry* (following paper in this issue).

Stone, A. L. (1964) *Biopolymers 2*, 315–325.

Stone, A. L. (1965) *Biopolymers 3*, 617–624.

Stone, A. L. (1969) *Biopolymers 7*, 173–188.

Stone, A. L. (1971) *Biopolymers 10*, 739–751.

Swann, D. A., & Caulfield, J. B. (1975) *Connect. Tissue Res. 4*, 31–39.

Toole, B. P., Biswas, C., & Gross, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 6299–6303.

Welsh, E. J., Rees, D. A., Morris, E. R., & Madden, J. K. (1980) *J. Mol. Biol. 138*, 375–382.

Zimm, B. H., & Crothers, D. M. (1962) *Proc. Natl. Acad. Sci. U.S.A. 48*, 905–911.