Characterization of Polysaccharide Conformation and Interactions by Circular Dichroism: Anomalous Chiroptical Effects in Hyaluronate Systems

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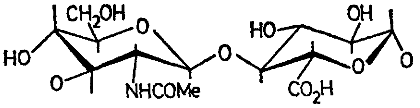
Summary Quantitative comparison with o.r.d. studies indicates that a low wavelength positive c.d. band previously reported for hyaluronate systems with enhanced viscoelastic character is a second-order effect of intramolecular structure, rather than of genuine chiroptical origin.

CHIROPTICAL methods show considerable scope for characterization of polysaccharide conformation and interactions.[1] The c.d. of carboxy and related chromophores (salt, ester, or amide) has proved particularly diagnostic.[2] Recently conformationally sensitive chiroptical behaviour has been reported[3] for hyaluronic acid (I), a polysaccharide of wide occurrence in animal tissues. This biopolymer may function in such diverse effects as the control of differentiation and level of hydration of biological tissues, and shows remarkable viscoelasticity, which is enhanced by partial suppression of ionisation, the greatest effect being at pH 2·5, where a viscoelastic 'putty' is formed.[4] The extent of intermolecular association has been correlated[3] with the magnitude of a previously unreported positive c.d. band in the $\pi\rightarrow\pi^*$ region below 200 nm, in addition to the well established 210 nm $n\rightarrow\pi^*$ negative band.



(I)

In attempting to trace the spectroscopic origin of this effect, however, we have observed marked discrepancies in the low-wavelength c.d. recorded on three different modern instruments (Cary 60, Cary 61, and Jasco J20). Indeed reproducible positive bands were obtained only on an instrument of the type used in the earlier investigation. Recent vacuum c.d. studies[5] show similar results. Furthermore, comparison with previously reported[3] optical rotatory dispersion data suggests that the $n \rightarrow \pi^*$ band alone is sufficient to account for the observed o.r.d. amplitude $[\phi]_{\max} - [\phi]_{\min}$, which for an isolated transition is 1·22 $[\theta]$ ($[\phi]$ and $[\theta]$ are o.r.d. molar rotation and c.d. molar ellipticity, respectively). In the present work we have therefore reinvestigated the chiroptical behaviour of hyaluronic acid, making use of the known relationships[6] between c.d. and o.r.d.

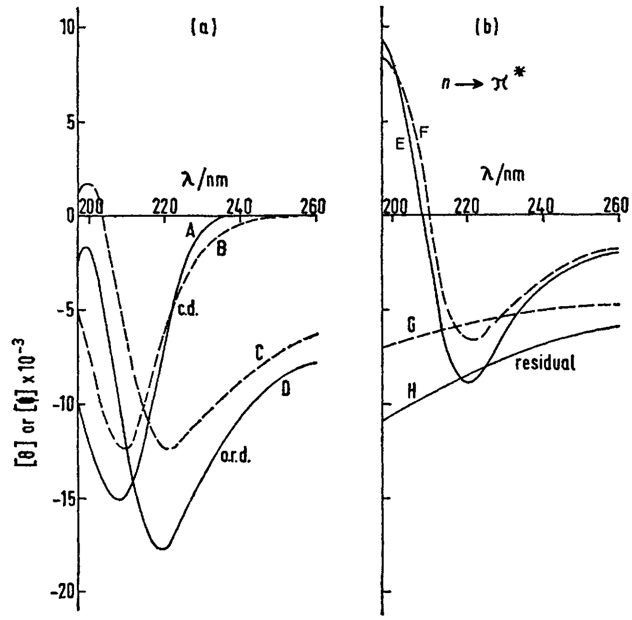


FIGURE. (a) C.d. (A and B) and o.r.d. (C and D) spectra of hyaluronate solution (——) and pH 2·5 putty (- - -). (b) Curves E and F: $n \rightarrow \pi^*$ contribution to hyaluronate o.r.d.; curves G and H: residual o.r.d. from all other transitions. (——, neutral solution; - - -, acid putty).

The Figure (a) shows both c.d. and o.r.d. of a neutral solution and acid putty. Over the wavelength range shown, these are in good agreement with previously reported spectra. Kronig–Kramers transform of the observed negative c.d. bands gives the o.r.d. contribution of the $n \rightarrow \pi^*$ region alone. Subtraction from the observed o.r.d. leaves only residual plain curves [Figure (b)], which Drude treatment suggests are centred in the far vacuum u.v., where transitions of the polysaccharide backbone are known to occur.[7] There is no indication of any detectable Cotton effect in the $\pi \rightarrow \pi^*$ region. We therefore conclude that the previously reported low wavelength c.d. effects do not have their origin in normal chiroptical behaviour.

They are, however, associated with the sol → putty conversion, and presumably reflect the development of internal structure. The danger of chiroptical artefacts, which may depend heavily on the exact optical layout of the instrument used, is well documented[8] for highly structured systems such as membranes,[9] suspensions,[10] and films.[11] The conclusion that we now report, that similar effects may arise for interacting biopolymers under conditions of extensive hydration, suggests the need for even greater caution in the application of chiroptical techniques to biological materials. In particular the internal consistency of o.r.d. and c.d. provides a useful criterion of the validity of chiroptical evidence.

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