though not, in general, significantly sensitive to the minor conformational variations involved in the final stages of structure refinement. (2) Special properties are predicted for the proline residues because they are spectroscopically distinct from the other (secondary) peptides. In structures such as the α/β structure, when they are in close proximity, they are able to develop a strong rotational couplet. The absence of such an effect from the circular-dichroism spectrum at 200nm suggests that they are not in fact close intrachain neighbours. (3) It is found that the rotatory properties are determined by the whole molecular conformation, and cannot be adequately treated as the sum of two half-molecules, even for a molecule with approximately twofold symmetry.

Specifically, it is found that, of the proposed structures, the only conformation that demonstrates strong negative $R_{\pi-\pi^*}$, with correct properties for the proline transitions, is that proposed by Hodgkin & Oughton (1957) and by Schwyzer (1958). The computed circular-dichroism properties are thus in conformity with the n.m.r. results in supporting this conformation for gramicidin S in solution.

- Balasubramanian, D. (1967). *J. Am. chem. Soc.* **89**, 5445.
 Bayley, P. M., Nielsen, E. B. & Schellman, J. A. (1969). *J. phys. Chem., Ithaca*, **73**, 228.
 DeSantis, P. & Liquori, A. M. (1971). *Biopolymers*, **10**, 699.
 Hodgkin, D. C. & Oughton, B. M. (1957). *Biochem. J.* **65**, 752.
 Laiken, S. L., Printz, M. P. & Craig, L. C. (1969a). *Biochemistry, Easton*, **8**, 519.
 Laiken, S. L., Printz, M. P. & Craig, L. C. (1969b). *J. biol. Chem.* **244**, 4454.
 Liquori, A. M., DeSantis, P., Kovacs, A. L. & Mazzarella, L. (1966). *Nature, Lond.*, **211**, 1039.
 Momany, F. A., Vanderkooi, G., Tuttle, R. W. & Scheraga, H. A. (1969). *Biochemistry, Easton*, **8**, 744.
 Quadrioglio, F. & Urry, D. W. (1967). *Biochem. biophys. Res. Commun.* **29**, 785.
 Ruttenberg, M. A., King, T. P. & Craig, L. C. (1965). *J. Am. chem. Soc.* **87**, 4196.
 Schwyzer, R. (1958). In *Ciba Found. Symp.: Amino Acids and Peptides with Anti-metabolic Activity*, p. 171. Ed. by Elliott, K. & Wolstenholme, G. E. W. London: J. and A. Churchill Ltd.
 Schwyzer, R. & Ludescher, U. (1968). *Biochemistry, Easton*, **7**, 2519.
 Scott, R. A., Vanderkooi, G., Tuttle, R. W., Shames, P. M. & Scheraga, H. A. (1967). *Proc. natn. Acad. Sci. U.S.A.* **58**, 2204.
 Stern, A., Gibbons, W. A. & Craig, L. C. (1968). *Proc. natn. Acad. Sci. U.S.A.* **61**, 734.
 Vanderkooi, G., Leach, S. J., Nemethy, G., Scott, R. A. & Scheraga, H. A. (1966). *Biochemistry, Easton*, **5**, 2991.

Circular Dichroism of Stacked Nucleotide Chromophores

By P. M. BAYLEY. (*National Institute for Medical Research, Mill Hill, London N.W.7, U.K.*)

The optical properties of diadenosine 5',5'-pyrophosphate (App5'A) differ from those of diadenosine 3',5'-monophosphate (ApA), indicating that the molecules have different conformations (Michelson, 1963; Massoulié & Michelson, 1964). In circular dichroism both molecules exhibit classical strong exciton splitting in the form of a positive couplet and the dichroism is conservative (Bayley, 1968; Scott & Zamecnik, 1969). However, for App5'A the amplitude of the effect is only 30% that of ApA, and the cross-over occurs at 267nm rather than 260nm.

The properties of ApA have been rationalized in terms of a right-handed stacked structure, with stacking angle of 36° as in stacked polynucleotides (Bush & Tinoco, 1967). A more refined treatment incorporates temperature-dependent oscillations (Davis & Tinoco, 1968). The variation of properties with stacking angle has been evaluated in detail (Schneider & Harris, 1969). However, these treatments appear to be unable to reproduce the distinctive properties of two adenine chromophores stacked as in the pyrophosphate. To generate a cross-over at 267nm in a conservative system, more than one $\pi-\pi^*$ transition must be included in the region of 260nm. The correlations of purine transitions with those of aromatic chromophores indicate that a second transition must be present under the intense 260nm adenine absorption band, though it is not resolved (Clark & Tinoco, 1965).

A formal model has been set up allowing the interaction of two chromophores, each containing two $\pi-\pi^*$ transitions (at 260nm and 267nm) oriented at an angle (β) close to 90°. By using a simple dipole approximation, the optical properties have been computed for the coupled system as a function of (1) the stacking angle (α), (2) small variations about 90° of the angle β and (3) parallel and antiparallel conformations. The distribution of oscillator strength is fixed at 80:20 for the 260nm and 267nm transitions respectively. Circular-dichroism profiles are computed by assuming a Gaussian bandwidth of 15nm for all transitions. Solutions are sought with characteristics of the App5'A spectrum.

With this model it can be shown that, with $\beta = 90^\circ$, no satisfactory results are obtained, since coupling of the 260nm transitions predominates. With $\beta \neq 90^\circ$, and with the 260nm transitions parallel ($\alpha = 0^\circ$) or perpendicular ($\alpha = 90^\circ$), the 260nm transitions still generate rotational strength, through the off-diagonal elements of the secular

matrix that couple the 267nm transitions, and through the 260–267nm off-diagonal elements. It is therefore important that the 267nm and 260nm transitions should be treated simultaneously and not in isolation.

Two regions have been found in which the properties of the model resemble those of App5'A. These are characterized by a stacking angle close to 0° and one close to 90°. In all of these the circular-dichroism profile derives from the overlap of all four transitions. With $\alpha \approx 0^\circ$ the main contributors are one of the 267nm transitions and a 260nm transition that has been shifted by the complex exciton interactions to approx. 265nm. With $\alpha \approx 90^\circ$ the profile derives from the resultant of two strong opposite bands close to 260nm with some rotational strength at 267nm. Of the two solutions the one with the low stacking angle would appear to be favoured, since it is the better candidate for the generation of strong hypochromism, as is shown by App5'A. This inference will be tested by including interactions with transitions located at 200nm.

- Bayley, P. M. (1968). *Abstr. I.U.P.A.C. 5th int. Symp., London*, no. E23.
 Bush, C. A. & Tinoco, I., jun. (1967). *J. molec. Biol.* **23**, 601.
 Clark, L. B. & Tinoco, I., jun. (1965). *J. Am. chem. Soc.* **87**, 11.
 Davis, R. C. & Tinoco, I., jun. (1968). *Biopolymers*, **6**, 223.
 Massoulié, J. & Michelson, A. M. (1964). *C. r. hebd. Séanc. Acad. Sci., Paris*, **259**, 2923.
 Michelson, A. M. (1963). *The Chemistry of Nucleosides and Nucleotides*, p. 447. New York: Academic Press Inc.
 Schneider, A. S. & Harris, R. A. (1969). *J. chem. Phys.* **50**, 5204.
 Scott, J. F. & Zameenik, P. C. (1969). *Proc. natn. Acad. Sci. U.S.A.* **64**, 1308.

A Refinement of the Structure of Lysozyme

By R. DIAMOND and M. LEVITT. (*Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.*)

Two techniques will be outlined. The first technique (due to R.D.) optimizes the interpretation of an electron-density map in terms of a flexible-chain molecule, and the second technique (due to M.L.) minimizes an energy function associated with the molecule. Collaborative use of the two techniques leads at once to co-ordinates that are stable with respect to both procedures to better than 0.1 Å root mean square. Remaining problems that will be discussed are concerned with the best treatment of indistinct regions of the electron-density map and with the empirical improvement of the energy parameters used in the second technique.

The Molecular Structure of Hyaluronic Acid

By E. D. T. ATKINS and J. K. SHEEHAN. (*H. H. Wills Physics Laboratory, University of Bristol, Royal Fort, Tyndall Avenue, Bristol BS8 1TL, U.K.*)

Hyaluronic acid exists naturally on a hydrated gel. It is the constituent of synovial fluid, which has the property of acting as a lubricant; it also occurs in the vitreous humour, where its function is probably to maintain the precise shape of the eye. Hyaluronic acid is a member of a small group of biologically important macromolecules known as the mucopolysaccharides. Chemically it is a regular unbranched polymer with a repeating unit of the type $(-N-G-)_n$ (where N is *N*-acetylglucosamine and G is glucuronic acid). The glycosidic linkages between *N*-acetylglucosamine and glucuronic acid and between glucuronic acid and *N*-acetylglucosamine are (1eq,4eq) and (1eq,3eq) respectively.

Recently we have obtained a number of oriented fibres and films of hyaluronic acid together with its salts with various univalent and bivalent ions.

From the X-ray fibre-type diffraction patterns obtained we have been able to elucidate the molecular structure of the free acid and its salts. Sodium hyaluronate, for example, may be crystallized in two distinct hexagonal unit cells, but in each case the molecular conformation of an individual chain is a threefold helix. In the free acid the conformation is tightened to a twofold helix.

We also suggest a reason for the alternate (1eq,3eq) and (1eq,4eq) glycosidic linkages. They are needed to position all the important substituent groups equally on both sides of the chain in the free acid form. The simpler (1→4)-linked hexosans [e.g. chitin (Carlstrom, 1957) and polymannuronic acid (Atkins, Mackie & Smolko, 1970)] need only one type of linkage to acquire the same features. On formation of a salt with a univalent ion a rotation occurs along the chain; this is partly due to steric hindrance of the cation but also has the advantage of distributing the ionic charge more evenly over the surface of the polymer.

The ability to crystallize the material in a number of forms enabling the chain conformation to be sampled in a variety of environments will help in predicting the molecular shape in the more dilute environment.

- Atkins, E. D. T., Mackie, W. & Smolko, E. E. (1970). *Nature, Lond.*, **225**, 626.
 Carlstrom, D. (1957). *J. biophys. biochem. Cytol.* **3**, 669.