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On the Structure of Nucleic Acids

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During the last few years, X-ray crystal structure determinations have been carried out of a number of decomposition products of the nucleic acids. Although these investigations by no means can be considered complete, the results so far obtained would appear to be important enough to justify a discussion of their contribution to an understanding of nucleic acid structure.

The investigations have been extended to pyrimidines [1,2], purines [3,4] and nucleosides [5,6], and have a bearing on nucleic acid structure from several points of view. Firstly, they give general stereochemical information on the nucleic acids, such as the mutual orientation of sugar and base, and bond angles and bond lengths. Secondly, the study of these crystal structures may provide information regards the forces acting between adjacent nucleotides. Extensive systems of hydrogen bonds are found in all the crystals studied, and such bonds must be expected to occur also in the nucleic acids, probably both within and between the molecules. The occurence of hydrogen bonding in nucleic acids has been suggested earlier from certain anomalies in the titration curves [7]. Thirdly, a comparison of the physical properties (*e.g.* density and refractive indices) of the nucleic acids with those of nucleosides and nucleotides with known structures may render it easier to estimate the structural significance of these properties. Thus the strong negative birefringence of sodium thymonucleate fibres [8] indicates that the pyrimidine and purine rings are far more nearly parallel in this compound than in the crystals of cytidine, which show positive birefringence [5]. A comparison of the densities, which are 1.63—1.65 g/cm^3 for the nucleic acids [9] and in the order of 1.60—1.75 g/cm^3 for the nucleotides and their sodium salts [10], indicates that the nucleotides are packed so as to be roughly equally close in the two cases.

The information obtained regarding the mutual orientation of the components of the nucleotides is of special importance, and will be discussed below.

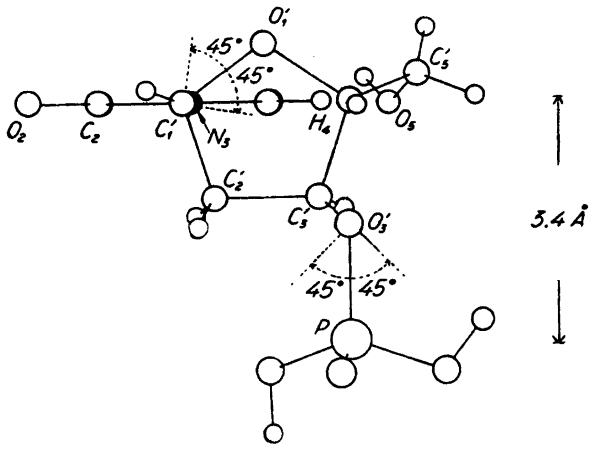

Fig. 1. A pyrimidine nucleotide of the "standard" configuration. The plane of the pyrimidine ring, as well as the bond N_3-C_1', is perpendicular to the plane of the paper.

THE STRUCTURE OF NUCLEOSIDES

In both the two nucleosides studied in some detail, cytidine [5] and 2',3'-*iso*-propylidene 3,5'-*cyclo*adenosine iodide [6], it is found that the bond connecting sugar and base (N-C_1') lies in the plane of the pyrimidine (purine) ring and makes tetrahedral angles with the adjacant ring bonds in the ribose ring. There would seem to be little reason to believe that the other nucleosides are different in this respect, and in the following it will be assumed that these stereochemical features are common to all nucleosides and nucleotides, whether of the ribose or the deoxyribose type [17]. The bond N-C_1' is found to be a single bond, and so rotation about this bond is possible. However, not all relative orientations of the sugar and base are equally feasible, as in many positions the hydrogen atoms at C_2' and C_3' come unfavourable near the atoms O_2 or H_4 of the pyrimidine, and N_3 or H_8 of the purine. The most favourable position would appear to be the one shown in Fig. 1, which is found in the crystal structure of cytidine. Here the pyrimidine is roughly perpendicular to the "plane" of the sugar (the furanose ring is not quite planar [5]) and the bond C_2'-C_3' approximately parallel to the plane of the base. There is, however, a certain range of favourable positions; the ribose ring may be rotated about 45° on either side of the position shown in Fig. 1 without bringing atoms of the ribose ring and the base too close together. For the purine nucleosides one would expect a similar structure, with N_3 approximately in the same position as O_2 in Fig. 1, the six-membered ring thus pointing away from the CH_2OH

group [17]. It should be noted that rotating the pyrimidine or purine 180° about the bond N-C_1' gives a structure which energetically would not appear to differ much from the one shown in Fig. 1.

THE STRUCTURE OF NUCLEOTIDES

No crystal structure determinations of nucleotides have been published, and there is no experimental evidence on the exact orientation of the phosphate group relative to the rest of the molecule. From a consideration of the van der Waals forces, however, it would appear that the positions within the 90° range shown in Fig. 1 probably are the most favourable ones, and that of these, the middle one with the bond P-O_3' roughly perpendicular to the plane of the pyrimidine (purine) is to be preferred. The distance from this plane to the phosphorous atom is about 3.4 Å. The relative position of $(OH)_5'$ and one of the phosphate oxygens may be very favourable to the formation of an intramolecular hydrogen bond.

In the following the structure shown in Fig. 1, and described above, will be called the *standard* nucleotide structure. Supposing that the free 3'-nucleotides have this configuration, the next question that naturally arises is the extent to which the nucleotides maintain this shape when they unite through phospho-ester linkages to form nucleic acids. Clearly the influence of neighbouring nucleotides may modify the mutual orientation of the components within the nucleotides to a certain extent. However, stereochemical considerations and the structure determinations carried out so far are in favour of the *standard* configuration, and until contradictory evidence has appeared, it would seem reasonable to assume that the nucleotides have roughly this shape in the nucleic acids. This point can probably be settled definitely only by a detailed X-ray structure determination of a number of nucleotides, or preferably polynucleotides.

MODELS OF THYMONUCLEIC ACID

Chemical investigations have shown, that in thymonucleic acid the nucleotides are linked together by phospho-ester linkages between hydroxyl groups at C_3' and C_5'. The chain is unbranched, or branched only to a small extent [7]. The molecules have the shape of long stiffish rods of diameter 15—20 Å [12]. The purine and pyrimidine rings are all nearly parallel, and are perpendicular to the long axis of the molecule, as was first pointed out by Signer, Hammarsten and Caspersson [13]. Astbury [9,11] obtained X-ray diagrams of fibres of sodium thymonucleate, the most dominating feature of which was a strong meridional

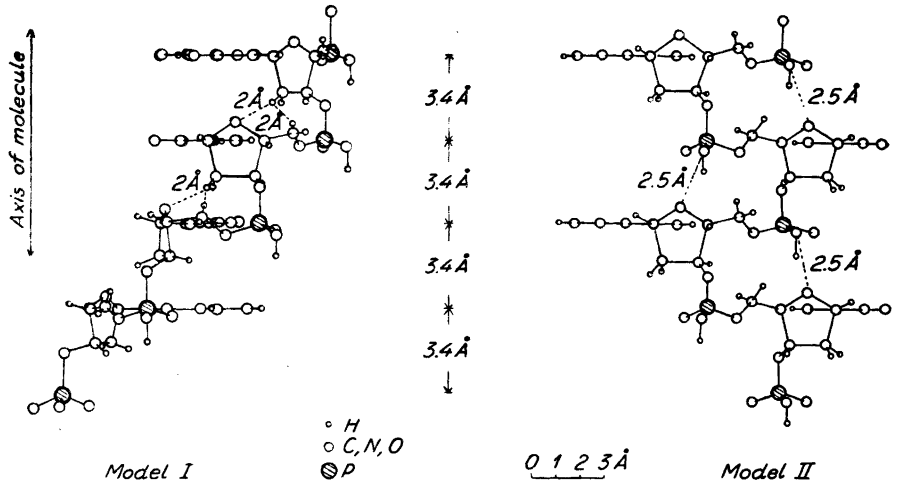

Fig. 2. Two models of thymonucleic acid based on nucleotides of the "standard" configuration. The planes of the purine and pyrimidine rings are perpendicular to the plane of the paper.

spacing of 3.4 Å (more accurately 3.3_4 Å). He also drew attention to the high density of the nucleic acids (1.63—1.65 g/cm^3), which shows that the nucleotides must be packed very closely in the polynucleotide. X-ray diagrams of specimens prepared in a different way show a far less pronounced 3.4 Å meridional reflexion [15].

Astbury [9] has proposed a model of thymonucleic acid, which is in agreement with the data given above. He associated the 3.4 Å spacing with the thickness of the nucleotides, assuming the nucleotides to be flattish and to stand out perpendicularly to the long axis of the molecule. The purine, pyrimidine and sugar rings were all believed to be flat and parallel, so that the structure is not consistent therefore with the results of the X-ray investigations of the nucleosides, which show that the sugar and base are nearly perpendicular to each other.

Two models which retain some of the essential features of the Astbury model, but are based on nucleotides of the *standard* configuration, are shown in Fig. 2. In contrast to the Astbury model, these two modifications of it have the planes of the sugar rings, as well as the bonds P-O_3', roughly parallel to the long axis of the molecule. Normal bond lengths and bond angles are maintained throughout the structure. Most atoms, the phosphorous atoms included, lie in planes 3.4 Å apart, thus explaining the strong 3.4 Å reflexion. It should be emphasized, that there is considerable freedom of rotation about

many of the single bonds in the structure, such as N-C_1', O_3'-C_3' and P-O_3', and considerable deviations from the *standard* configuration are possible within the general structural scheme in Fig. 2. The bonds P-O_3' may for example not be exactly parallel to the long axis of the molecule, and the sugar rings may be slightly twisted. According to Fraser and Fraser [14] a slight reorientation of the phosphate group is necessary to explain the results of their recent infra-red measurements, although the models in Fig. 2 in general fit their data well.

In *model I* the pyrimidine and purine rings are piled in a central column directly on top of each other, 3.4 Å apart, with the ribose rings and the phosphate groups in a spiral enclosing the column. Van der Waals attraction between the flat rings in the column holds the structure together. However, it has not been found possible to place the bases directly on top of each other without bringing some of the other atoms in successive nucleotides too near one another. As indicated in the figure, some of the distances from the hydrogen atoms at C_2' to neighbouring atoms are as short as about 2 Å, which is unfavourable. On the other hand, these steric distortions are not so serious that the model must be rejected, and they are not as great as those involved in the Astbury model.

The relative position of the electronegative groups in model I excludes the direct formation of intra-molecular hydrogen bonds. By means of water molecules, however, such bonding may be assumed to take place between electronegative atoms in successive pyrimidines and purines, as well as to the oxygen atoms of the phosphate groups. Astbury [9] has pointed out that water molecules must play an important part in the stability of crystalline aggregates of sodium thymonucleate.

The spiral in structure I repeats itself after eight nucleotides, the bonds N-C_1' in successive nucleotides making angles of about 45° with one another.

In *model II* the ribose rings and the phosphate groups form a flattish central column, from which the purines and the pyrimidines stand out perpendicularly. Successive bases are pointing outwards in opposite directions, all the bonds N-C_1' being roughly parallel, and there is one base directly above another at a distance of 6.8 Å. There are thus no forces acting between purines and pyrimidines; the whole molecule must be held together by interactions between atoms in the pentose rings and the phosphate groups, which are packed very efficiently in a compact sheet. Only very small steric distortions occur. The oxygen atom of the ribose ring and one of the oxygens of the phosphate group are 2.5—3.0 Å apart, and in the case of the free acid the molecule may possibly be stabilized by hydrogen bonds between these atoms. Hydrogen bonds to the ring oxygen atoms do not occur in crystals of cytidine [5], but appear to exist in crystals of *α*-D-glucose [16]. It will be seen that neighbouring molecules of the structure II

may be closely interleaved, giving a packing which is close enough to account for the high density.

These two models are chosen as examples to illustrate the mode of packing and the possible intra-molecular forces in models of thymonucleic acid based on *standard* shape nucleotides. As emphasized earlier, there is freedom of rotation about the P-O_3' bonds, and structure II may actually be derived from structure I solely by rotation about these bonds. A great number of intermediate structures, as well as combinations of I and II may be derived in this way. However, not all these models are equally feasible; in many of the structures van der Waals repulsive forces will introduce strains, and the molecules will be so irregular in shape that it would appear difficult to pack them closely. This kind of evidence is, of course, by no means conclusive, and on the whole the available evidence about the internal structure of thymonucleic acid does not permit a choice to be made at this stage between the structures I and II, or some of their intermediates.

Astbury observed that the true repeat distance along the fibre axis was much greater than 3.4 Å, probably eight or sixteen times as great, a finding which he associated with the tetra-nucleotide hypothesis favoured at that time. The models I and II may both be made to have repeat units of this magnitude by appropriate choice of the sequence of the nucleotides.

It has been assumed above, that the nucleotides have the *standard* configuration in the nucleic acids, which means that the phosphorous atom falls approximately in the plane of the base in the nucleotide below. By rotation about the bonds N-C_1' and C_3'-O_3' the phosphorous atom may be brought into the plane of its own purine (pyrimidine). Models based on nucleotides of this shape are also possible, and cannot be ruled out in general, but as we have seen above, the available evidence favours the *standard* configuration for the nucleotides.

SUMMARY

The information obtained on the structure of the nucleic acids from crystal structure determinations of nucleosides and related compounds has been discussed. A probable configuration of the nucleotides is suggested, and modifications of the Astbury model of thymonucleic acid proposed. In these new models the pentose rings are roughly parallel to the long axis of the molecule.

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