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Triple-Stranded Polynucleotide Helix Containing Only Purine Bases

Abstract. *The structure of the complex involving one polyadenylic acid and two polyinosinic acid chains has been determined by x-ray diffraction. The three coaxial, helical chains have conformations like conventional RNA double helices despite the absence of purine-pyrimidine pairing. Formation of hypoxanthine pairs in codon-anticodon interactions therefore requires only trivial changes in the conformation of a standard nucleotide. Evolution of the contemporary genetic code involving purine-pyrimidine complementarity from a primeval code with only adenine-hypoxanthine pairing would have been possible without major discontinuities in molecular geometry.*

We have obtained good-quality x-ray diffraction patterns (Fig. 1) from fibers of poly(I)·poly(A)·poly(I) (*1*) and have developed a detailed molecular model that corresponds closely to the diffracted intensity distribution. The molecule contains hydrogen-bonded base triplets that consist of two kinds of doubly hydrogen-bonded adenine-hypoxanthine pairs (Fig. 2a) and is of interest for a number of reasons. Its structure shows that very little change in the conformation angles of a nucleotide in a conventional RNA (purine-pyrimidine pairs) is required to accommodate such nonstandard pairs. Adenine-hypoxanthine pairs occur in codon-anticodon interactions (*2*), but the (A·I) pair (*1*) (Fig. 2a) that has the same interglycosidic link distance (10.8 Å) as a Watson-Crick pair is not, we think, as good a candidate for the "wobble" base-pairing as the (A·I) pair with the longer interglycosidic link distance (13.0 Å). It has also been suggested that adenosine phosphate and its deaminated derivative, inosine phosphate, are likely to have been the most abundant nucleotides synthesized under prebiotic conditions and that, therefore, the self-replicating precursor of the modern nucleic acids might have contained only adenine and hypoxanthine bases (*3*). Our studies suggest that nucleic acid double helices containing only the longer (A·I) pairs could well have evolved to contemporary species with purine-pyrimidine pairs with no major discontinuity of molecular geometry.

Although the axial rise per residue (3.3 Å) of the poly(I)·poly(A)·poly(I) helix is reminiscent of *B*-DNA, which has 3-*exo*-puckered furanose rings (*4*), the general intensity distribution of Fig. 1 is like that from all members of the *A* family of polynucleotide structures (*5*). We indeed find that all three chains are right-handed helical structures that have 3-*endo*-puckered furanose rings and very similar chain conformation angles (Table 1), none of which differs by more than 13° from the average values (*6*, *7*) found in standard double helices of the *A* type.

Arnott and Hukins (*7*) and Sundaralingam (*8*) have pointed out the remarkable extent to which conformations preferred in mononucleotides are preserved in the nucleotide residues of DNA and RNA double helices. This conformational conservatism extends to this rather different triple-stranded poly(purine) nucleotide system. From another viewpoint, it means that one common nucleotide shape is sufficiently flexible, through unexceptional variations in conformation angles, to allow both complementary purine-pyrimidine pairing and purine-purine-purine triplet formation in stable helical structures.

In the model (Fig. 2), the poly(I_1) chain is antiparallel to the poly(A) chain, and the poly(I_2) is parallel (*9*). Both the antiparallel and the parallel

Table 1. Conformation angles in poly(I)·poly(A)·poly(I) compared with appropriate average values and estimated standard deviations (in parentheses) of nucleic acid double helices. The relevant conformation angles are defined in (*5–7*, *11*) and shown in Fig. 2b. It was convenient to assume initially that poly(I_1) and poly(A) have identical conformation angles. Relaxing this requirement does not lead to an appreciably better structure.

Angle	I_1 and A (deg)	I_2 (deg)	Double helices (deg)
φ	− 66	− 61	− 69 (18)
ψ	− 73	− 57	− 69 (18)
θ	− 177	− 172	− 166 (18)
ξ	+ 63	+ 45	+ 50 (6)
σ	+ 79	+ 79	+ 83
ω	− 160	− 163	− 170 (22)
χ	+ 80	+ 83	+ 80 (9)

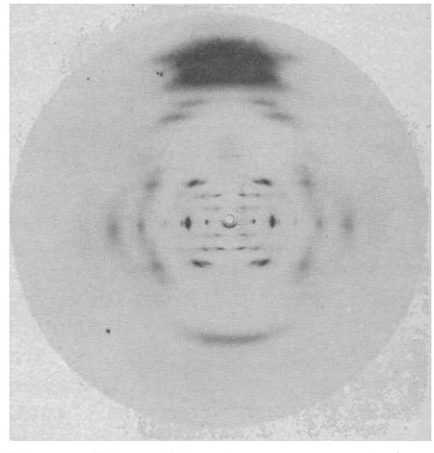

Fig. 1. Fiber diffraction pattern of the sodium salt of poly(I)·poly(A)·poly(I). The fiber was tilted 14° to the incident (Cu K_α) x-ray beam and maintained at 92 percent relative humidity during the exposure. The pattern corresponds to a helical structure with an axial rise per residue of 3.29 Å. The triple helices are packed on a hexagonal net of spacing 23.7 Å. Up-pointing and down-pointing triple helices occur randomly in the molecular sites, with the result that continuous intensity streaks accompany the sharper Bragg reflections. There is also "screw disorder" (*12*) that results in continuous diffraction in all but the center of the pattern.

poly(A)·poly(I) double helices are as slender as a standard RNA helix with purine-pyrimidine pairs. The effective diameter of the triple helix (23.7 Å) is only slightly greater than that of *A'*-RNA (23.2 Å) (*10*). The poly(A)·poly(I_2) double helix that is part of the triple-stranded complex has a distance between glycosidic carbons (Fig. 2a) that is almost the same (10.8 Å) as in a Watson-Crick pair, but its parallel chains preclude it as a model for both wobble base-pairing and an ancestral nucleic acid double helix. Despite the long distance (13.0 Å) between the glycosidic carbons (Fig. 2a), the antiparallel poly(A)·poly(I_1) double helix is remarkably similar in overall dimensions and in individual conformation angles to *A*- and *A'*-RNA (*11*). It is therefore not difficult to accept the replacement of standard pairs by these A·I pairs either in the present-day genetic machinery (codon-anticodon wobble interactions) or in a primitive situation in which adenosine and inosine phosphates were the main nucleic acid materials available.

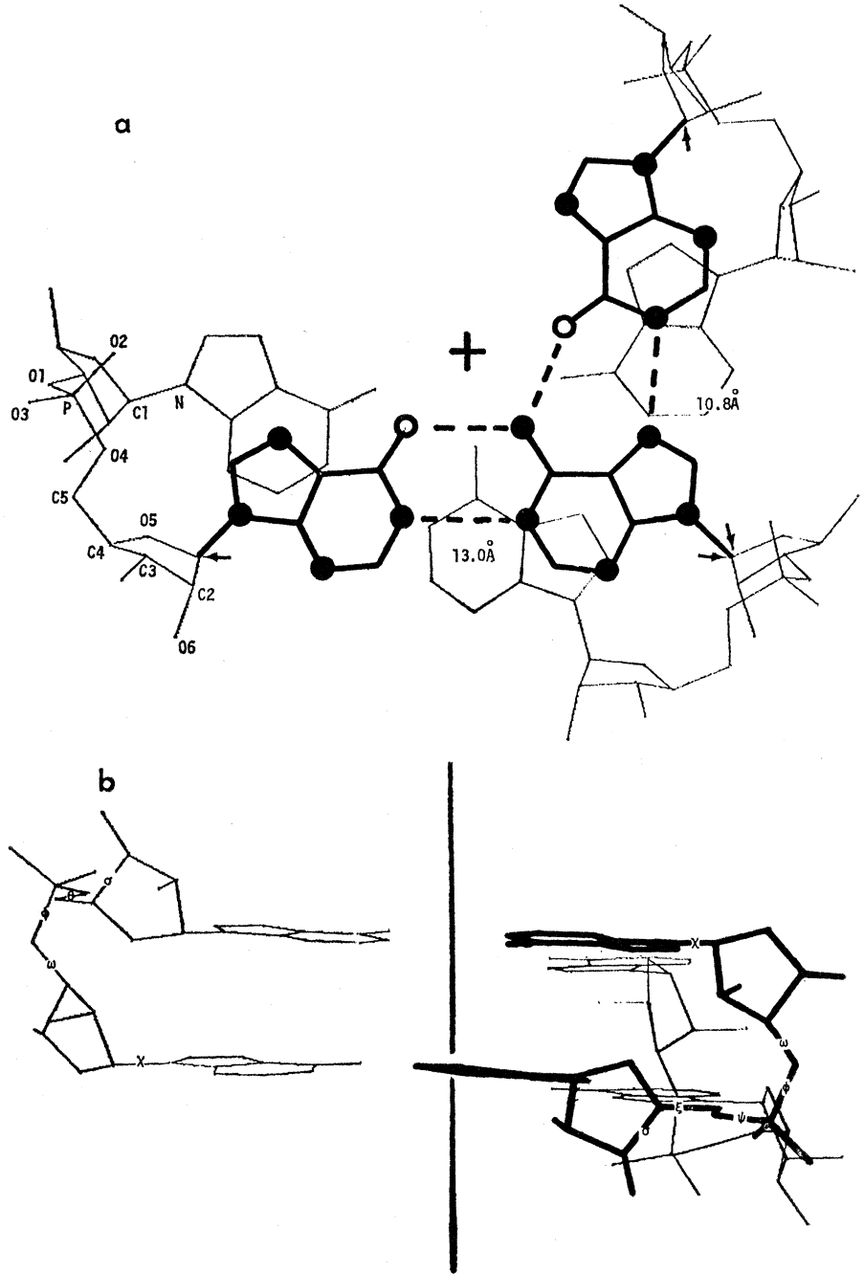

Fig. 2. Projections of part of the poly(I)·poly(A)·poly(I) structure (a) down the helix axis and (b) perpendicular to it. The helix axis is represented by a cross in (a) and a line in (b). Oxygen and nitrogen atoms of the upper bases (bold lines) in (a) are indicated by unfilled and filled circles, respectively. Hydrogen bonds are shown by the dashed lines. Interglycosidic link distances (between arrows) are also given. To distinguish between the two kinds of hydrogen-bonded hypoxanthines, we consider the hypoxanthine on the left to be I_1 and that on the right to be I_2. The structure was obtained by "linked-atom" molecular model building that preserved standard bond lengths and bond angles while contriving good fitting of observed and calculated diffraction (*13*) by variation of conformation angles only. The purine stacks in all three chains are very similar. Carbon, phosphorus, and oxygen atoms are labeled for the ribose phosphate at the left in (a), and conformation angles are shown in (b).

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References and Notes

1. We use poly(I) · poly(A) · poly(I) to indicate the three-chain polymer complex involving polyadenylic acid [poly(A)] and polyinosinic acid [poly(I)], and (A·I) to indicate a doubly hydrogen-bonded base-pairing between adenine and hypoxanthine. We obtained poly(I) · poly(A) · poly(I) by dissolving the homopolymers (0.25 mg/ml) in dilute (0.05*M*) sodium chloride solution buffered at *p*H 7.4, then mixing the solutions in the appropriate molar ratio, and finally, after 12 hours at 4°C, precipitating the complex with two volumes of ethanol. We determined the conditions for obtaining the desired stoichiometry from spectrophotometric mixing curves [G. Felsenfeld, D. R. Davies, A. Rich, *J. Amer. Chem. Soc.* **79**, 2023 (1957)] and confirmed the composition of the complex by analyzing the alkaline hydrolyzate of a sample [J. R. Fresco and B. M. Alberts, *Proc. Nat. Acad. Sci. U.S.A.* **46**, 311 (1960)].
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13. Standard bond lengths and bond angles are the average values found in monomers. For a recent survey of these, see (*6*). The linked-atom least-squares procedures for refining nucleic acid structures using conformation angles as the variable have been described by S. Arnott, S. D. Dover, and A. J. Wonacott [*Acta Crystallogr. Sect. B* **25**, 2192 (1969)] and in (*5*).

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