stable plateau voltage (*15*) was achieved because a gradual decrease in the light-activated Na^+ conductance was compensated for by a gradual decrease in the voltage-dependent K^+ conductance. If this compensation did not occur, the plateau voltage would slowly droop, and a steady illumination might be falsely perceived as a slowly dimming one (*16*).

One mechanism by which light might lower the K^+ conductance is reduction of intracellular *p*H (pH_i). Potassium channels in squid axon (*17*) are blocked by protonation of a site with an apparent *p*K of 6.9. A large light-induced reduction of pH_i occurs in the barnacle photoreceptor (*18*). In *Limulus*, however, measurements of pH_i made with phenol red indicate that the average pH_i of the cell is not significantly affected by light (*19*). Light does cause a large increase (*20*) in Ca_i^{2+}, and injection of Ca^{2+} causes a decrease in net outward current (*3*). This evidence suggests that Ca^{2+} might reduce the K^+ conductance, though there is little precedent for this in other preparations (*21*). Further work will be required to determine whether the K^+ conductance in *Limulus* is modulated by pH_i, Ca_i^{2+}, or some other cytoplasmic factor.

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3. J. Pepose and J. Lisman [*J. Gen. Physiol.* **71**, 101 (1978)] showed that the steady-state outward current in *Limulus* is carried by a single class of K^+ channels that appears to be directly gated by voltage, rather than activated by Ca^{2+}. (Even though there is no direct evidence of a Ca-activated K^+ current in *Limulus*, it is impossible to rule out a minor contribution from such a component.) They further showed that the K^+ conductance is similar to that of the squid K^+ channel pharmacologically. Additional evidence bearing on this similarity is that, as in the squid channel, there is an *e*-fold change in conductance for each ~ 5-mV change in voltage (data not shown). Furthermore, the closing of the channels, as revealed by tail currents, follows a single exponential that decreases with increasing negativity, and the instantaneous current-voltage curve is linear (Fig. 2a).
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9. In four of ten cells, there was no change in the voltage of half-maximal activation of outward current ($V_{1/2}$). In one cell, $V_{1/2}$ was made more negative, and in five others, more positive. The average shift for ten cells was +4.3 ± 3.9 mV.
10. Conductances do not change during a rapid (instantaneous) change in voltage. Thus, an instantaneous current-voltage curve measured just after repolarization provides a measure of the conductances activated (at steady state) by depolarization.
11. The observed change in the reversal potential was +11 mV. The change predicted by the Nernst equation is +28 mV. This discrepancy can be explained if the channel is not perfectly selective for K^+, and has a permeability ratio of 16 : 1 for K^+ to Na^+.
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15. We used interrupted flashes so that the changes in I_K and I_{Na} could both be observed. Experiments reported by G. Fain and J. Lisman (*Prog. Biophys. Mol. Biol.*, in press) in which the Na^+ current was observed during a long uninterrupted flash also show a progressive decline in the magnitude of I_{Na} with no decline in the plateau voltage response.
16. The mechanism by which the ventral photoreceptors send signals to the brain is not understood. In the lateral eye, which has many of the properties of the ventral eye, signaling is accomplished by changes in action potential frequency. The frequency of spikes is sensitive to voltage [0.77 spike per second per millivolt was measured by M. Fuortes, *J. Physiol. (London)* **148**, 14 (1959)].
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22. The conductance turns off as a single exponential. The τ for a given repolarization is not changed by illumination.
23. We thank B. Meech, G. Fain, and A. Szent-Györgyi for commenting on the manuscript.
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Absence of Correlation Between Base-Pair Sequence and RNA Conformation

Abstract. *A survey of all available double-stranded RNA crystal structures shows that there is a considerable range of variation in local conformation of a given base-pair doublet, but that there is no significant correlation between base-pair sequence and RNA local conformation.*

The presence or absence of correlation between base-pair sequence and DNA-RNA conformation at the local level is important for understanding protein–nucleic acid interaction, one of the most central processes in all cells. Until now it was not possible to answer the question owing to lack of a large enough data base for statistical studies. In past years there has been an effort to experimentally determine the conformational variation of double helical nucleic acids by various optical spectroscopic techniques such as ultraviolet circular dichroism and infrared linear dichroism. Such effort resulted in several indirect pieces of evidence that the conformation of double-stranded DNA (dsDNA) depends on solution conditions and base sequence (*1–4*). However, these techniques suffer from the fact that they measure average properties of entire molecules rather than local properties such as local conformation. Thus no structural details can be derived.

Until recently, structural details of double-stranded polynucleotides have been derived exclusively from x-ray fiber diffraction data, the interpretation of which depends on the assumption that all nucleotides along a polymer chain have the same conformation. Like the optical techniques mentioned above, this technique gives information about the average structure only. Thus the three-dimensional structural model derived from this technique leaves the impression of extremely regular structures of dsDNA and dsRNA. Local heterogeneity in conformation which must exist is lost in these artificially uniform structures.

Recent determination of three-dimensional structures of dsDNA model compounds (*5–9*) provides the most specific and detailed support to the hypothesis that dsDNA structures do not have regular conformation throughout their entire length, but that the local conformation may be significantly different from the DNA-B form depending on the particular base sequence in that region and on the solution conditions. These self-complementary structures, dCGCGCG (C, cytosine; G, guanine) (*5*), dCGCG (*6, 7*), dATAT (A, adenine; T, thymine) (*8*),

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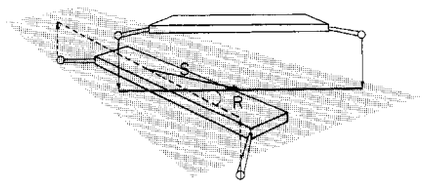

Fig. 1. The relative position of two adjacent base pairs is represented by projection of C1′ atoms onto a common plane between the two base-pair planes. This common plane is the average of the two base-pair planes calculated by the least squares method. The C1′ atoms from the two glycosyl bonds of the top base pair are projected onto this common plane, the projected points are connected, and a midpoint is defined. The same operation is done for the bottom base pair. The angle of intersection of these two lines is defined as the on-plane rotation angle (R). For left-handed helices, the values in general will be negative. The relative translation of these two base pairs is depicted by a vector connecting the two midpoints of the two projected lines. The magnitude of that vector is defined as the on-plane sliding distance (S).

and dCGCGAATTCGCG (*9*), may reflect the range of variability in dsDNA conformation. In the dATAT structure it was shown that the deoxy-sugar conformations alternate along the chain; in the dCGCGCG and dCGCG structures, not only the sugar conformation but also the glycosyl bond conformation alternates, thus generating an overall left-handed double helix; and in dCGCGAATTCGCG the overall structure is similar to the DNA-B form, but the local conformation varies widely, perhaps in a sequence-dependent manner (*10*).

A natural question arising from this series of observations is whether such conformational variability exists in dsRNA as well. Here we now have a much wider crystallographic data base (in sequence variety) available than for dsDNA discussed above. In this report, we make a detailed conformational analysis of crystal structures of the four double helical portions of one transfer RNA (17 base-pair doublets) and four dsRNA model compounds (four base-pair doublets) to test the hypothesis of conformational variability as a function of base sequence in dsRNA. Information obtained from x-ray fiber diffraction or optical spectroscopic methods was not used in our study for reasons stated above.

A complete description of nucleic acid conformation can be given by seven torsion angles per nucleotide (six for the backbone bonds and one for the glycosyl bond). Examination of these angles as a function of base-pair sequence shows no apparent correlation. It may be that these conventional torsion angles do not reflect the local interaction between base pairs in a simple manner. For dsRNA and dsDNA, it is therefore more convenient and direct, in physical meaning, to describe the local conformation of double helical nucleic acids in terms of the relative position of two adjacent base pairs (Fig. 1). Implicit in the above statement is that the conformation around a base pair is mostly dependent on interaction with its immediate neighbors, and that the base-pair twist is small, say, less than 15°.

The on-plane rotation angles (R) and the on-plane sliding distances (S) for all RNA base-pair doublets whose crystal structures are known are summarized in Fig. 2, a and b. It is evident from these two figures that, where comparison is

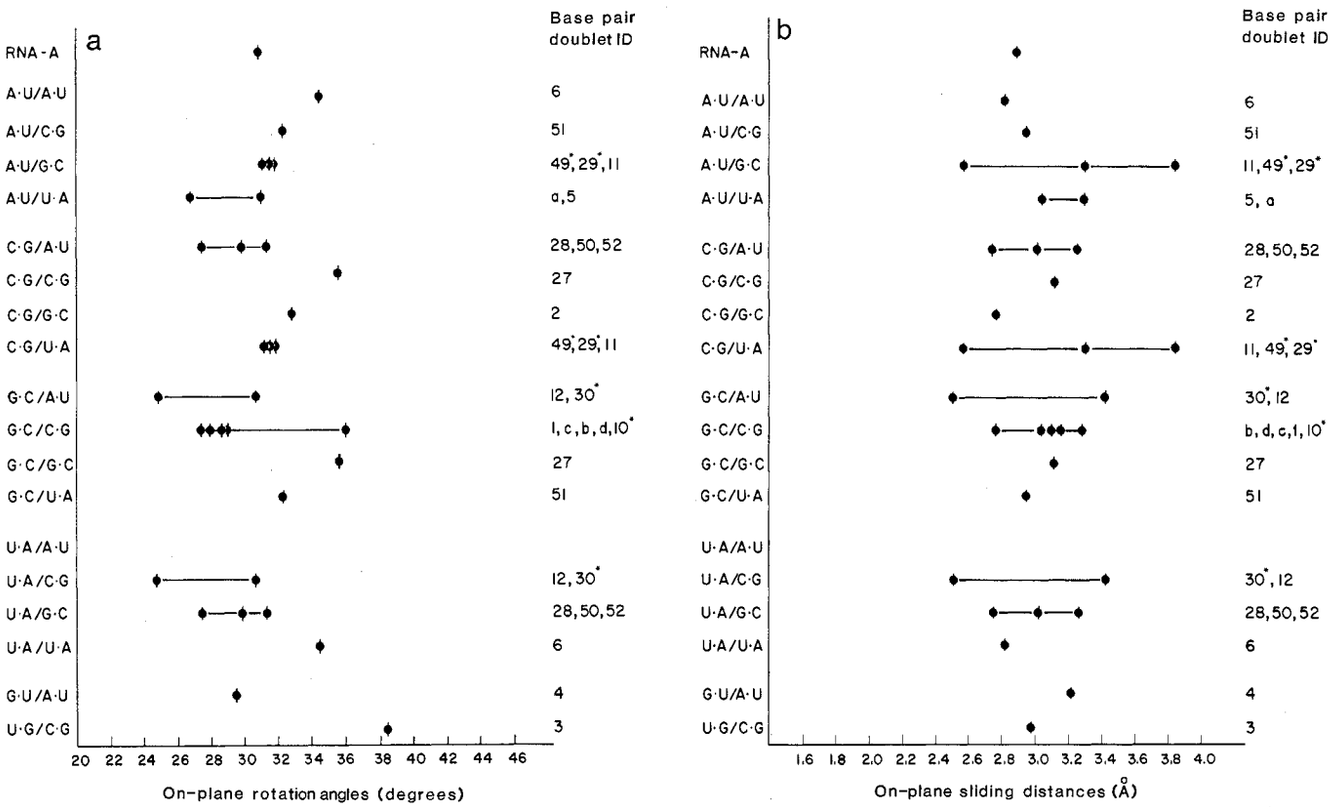

Fig. 2.(a) Observed on-plane rotation angles as defined in Fig. 1 are plotted against observed base-pair sequences. A particular base-pair doublet sequence indicated on the ordinate, for example, C·G/A·U (U. uracil), stands for a C·G base pair on top of an A·U base pair where each strand sequence, from 5′ to 3′ direction, will read C-A on one strand and U-G on the second strand. The base-pair doublet identification on the right-hand side is the lowest sequence number of the four bases involved in yeast phenylalanine tRNA (*13*). The *a*, *b*, *c*, and *d* are from self-complementary dinucleoside phosphate structures: *a* is from ApU (*14*), *b* is from the sodium salt of GpC (*15*), and *c* and *d* are from the calcium salts of GpC (*16*). The asterisk indicates that one of the bases involved in the base-pair doublet is a modified base. (b) The on-plane sliding distances as defined in Fig. 1 are plotted as a function of the sequence of two adjacent base pairs. The ordinate and base-pair identifications are the same as in (a).

possible, the conformational variation between two base-pair doublets of the same base sequence is similar to or sometimes greater than the variation between those with different base sequences. As an example (see Fig. 2, a and b), a base-pair doublet, G·C followed by A·U, has been observed twice in the crystal structure of yeast phenylalanine transfer RNA. The base-pair on-plane rotation angle in these two cases differs by about 6° and the on-plane sliding distance by 0.9 Å. Both are of the same order of magnitude as the total average values of all the base-pair sequences found in known crystal structures of double helical RNA, the average values being 7° and 0.8 Å respectively. In some cases, such as A · U followed by G · C, on-plane rotation angles of three base-pair doublets are very similar, suggesting a strong apparent correlation (Fig. 2a), but the on-plane sliding distances for the same three base-pair doublets vary widely (Fig. 2b).

Despite such wide variation in on-plane rotation angles and sliding distances of base-pair doublets, there has never been a drastic conformational difference such as was found in dsDNA crystal structures, where the purine-pyrimidine sequence appears to have a tendency toward mixed sugar conformations and, in one case, alternating glycosyl bond conformation as well.

In summary, the examination of 21 base-pair doublets found in crystal structures reveals that local conformations in general are not directly correlated to the base-pair sequence in dsRNA (of course we cannot claim this for the cases with only one example, such as A·U/A·U). This observation is in contrast to the finding that dsDNA appears to be significantly dependent on both the base-pair sequence and solution conditions. These analyses further suggest that (i) the backbone conformation of dsRNA is less flexible than that of dsDNA, (ii) although there is a large restricted variation of backbone conformation of dsRNA, all of these may be described as belonging to the A form (*11*); and (iii) the base-pair sequence, thus the base-base interaction and base-solvent interaction, can induce drastic conformational changes of the dsDNA backbone but not of the dsRNA backbone. This restricted conformational variability of dsRNA within the overall frame of the dsRNA-A form is probably one of the reasons that RNA, but not DNA, is used to build structural molecules such as transfer RNA and ribosomal RNA by all living cells. From a physical point of view, the O2′-hydroxyl group of RNA is probably playing double roles of sterically restricting the variation of backbone conformation and providing additional potential for hydrogen bond formation for structural rigidity, as was observed in the tertiary structure of yeast phenylalanine transfer RNA (*12*).

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Human Muscle Phosphoglycerate Mutase Deficiency: Newly Discovered Metabolic Myopathy

Abstract. *Muscle phosphoglycerate mutase activity was decreased (5.7 percent of the lowest control value) in a 52-year-old man with intolerance for strenuous exercise and recurrent pigmenturia since adolescence. All of the other enzymes of glycolysis had normal activities, and glycogen concentration was normal. Electrophoretic, heat lability, and mercury inhibition studies showed that the small residual activity in the patient's muscle was represented by the brain (BB) isoenzyme of phosphoglycerate mutase, suggesting a genetic defect of the M subunit which predominates in normal muscle. The prevalence of the BB isoenzyme in other tissues, including muscle culture, may explain why symptoms were confined to muscle.*

Cramps, myoglobinuria, and intolerance for strenuous exercise characterize the clinical picture of muscle phosphorylase and phosphofructokinase deficiencies (*1*). Phosphofructokinase deficiency is the only known genetic defect of the glycolytic pathway in human muscle. We now report a defect of muscle phosphoglycerate mutase (PGAM) (E.C. 2.7.5.3) in a patient with symptoms similar to those of phosphofructokinase deficiency. Starting in adolescence, this man noted muscle pain, "stiffness," and weakness after 15 to 30 minutes of intense exercise. The episodes were often followed by pigmenturia, but the urine was never examined for myoglobin. Despite these problems, he led a relatively normal life, served in the army, then worked as a security guard. At age 52, results of a physical examination were normal except for gouty tophi at the wrists and elbows; strength was normal. Severe atherosclerosis of the coronary arteries was present. On two occasions, ischemic exercise of the forearm was followed by an abnormally small rise of venous lactate; histochemical and ultrastructural studies of a muscle biopsy indicated a mild increase of glycogen (*2*).

The clinical syndrome and the minimal increase of lactate after ischemic exercise suggested muscle phosphorylase or phosphofructokinase deficiency, but both of these enzyme activities (*3*) were normal in a muscle extract (Table 1). Studies of anaerobic glycolysis in vitro (*3*) showed decreased lactate production by the patient's muscle, with glycogen or hexose-phosphate glycolytic intermediates from glucose 1-phosphate to fructose 1,6-diphosphate. This suggested a defect below the phosphofructokinase reaction (Fig. 1), which was confirmed by measurement of individual enzymes of glycolysis (*4*) (Table 1). All enzyme activities were normal except that of PGAM, which had a mean value of 3.6 percent of the normal mean. Although the range of PGAM activity in normal human muscle is wide (253 to 588 μmoles of 3-phosphoglycerate converted per

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