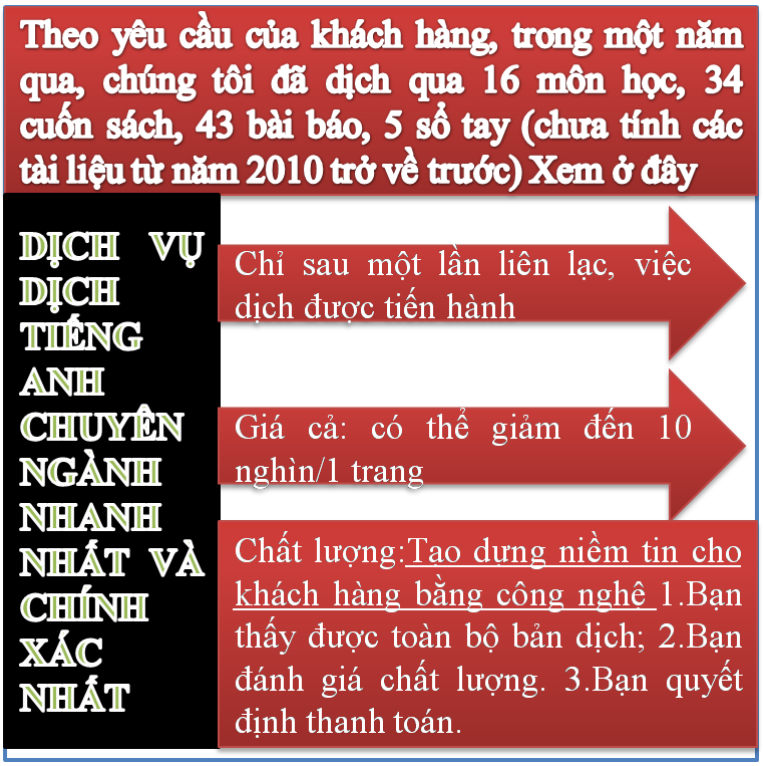

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2’endo conformation. Both these families are right-handed helices. The third family is the left-handed Z-AND. The main differences between the B and A forms DNA are in the conformation of the sugar ring. This dramatic change in structure can be understood by recognizing the importance of the changes induced on the sugar-photphate backbone (Fig. 16b); the distance between two successive phosphate atom on the AND chain changes from 0.70 nm in the B form to 0.59 nm in the A form. This change greatly affects the shape of the helix (Fig. 30). Although the large groove of B-DNA is about twice as deep as the small one, in A-DNA the two grooves are essentially of similar size. The depth of the grooves in A-DNA is, however, very different: the large groove is shallow, and the small one is very deep. This is due to the change of the position of the helix axis in the base pair. In B-DNA the axis passes through the base pair (close to the N^1 position of the purine; Fig.31a), whereas in A-DNA the helix axis lays astride in the large groove. The inclination of the base pair relative to the helix axis is directly related to these changes. Although the base are essentially perpendicular in B-DNA, they are inclined by about 15^o in A-DNA (fig. 30). The fiber diffraction data also established a direct relationship between the axial rise and the base tilt (Fig. 31b).	Cấu dạng (cấu tạo, hình dạng) 2'endo. Cả hai họ này đều xoắn phải. Họ thứ ba là DNA dạng Z xoắn trái. Sự khác biệt chính giữa DNA dạng B và A ở chổ cấu tạo của vòng đường. Chúng ta có thể hiểu được sự thay đổi đáng kể về mặt cấu trúc này qua việc nhận thức tầm quan trọng của những thay đổi gây ra trên chuỗi (mạch khung) đường-photphate (Hình 16b), khoảng cách giữa hai nguyên tử phosphate liên tiếp trên chuỗi DNA thay đổi từ 0,70 nm ở dạng B đến 0,59 nm ở dạng A.Sự thay đổi này ảnh hưởng rất lớn đến hình dạng của cấu trúc xoắn ốc (Hình 30). Mặc dù rãnh lớn của DNA dạng B sâu gấp hai lần rãnh nhỏ, trong DNA dạng A hai rãnh chủ yếu có cùng kích thước. Tuy nhiên, độ sâu của các rãnh trong DNA dạng A rất khác nhau: các rãnh lớn nông, và rãnh nhỏ rất sâu.Điều này là do sự thay đổi vị trí của trục xoắn trong cặp base. Trong DNA dạng B, trục đi qua cặp base (gần vị trí ^1N của purine; Fig.31a), trong khi đó trong DNA dạng A trục xoắn nằm chắn ngang trong rãnh lớn. Độ nghiêng của các cặp base đối với với trục xoắn liên quan trực tiếp đến những thay đổi này. Mặc dù, về cơ bản, base vuông góc trong DNA dạng B, chúng nghiêng khoảng 15^o trong DNA dạng A (Hình30). Các dữ liệu nhiễu xạ sợi cũng thiết lập một hệ thức trực tiếp giữa sự nâng trục và độ nghiêng của

In the last few years progress in technology and computational techniques have permitted the discovery of numerous new structure. The search for such structures has been stimulated by the discovery of Z-DNA and the awareness that alternating poly (puRine) poly (pYrimidine) [poly (R) poly (Y)] sequences must have very characteristic features. The term wrinkled was introduced for these helices. They are characterized by a repeating unit that is not one base pair, as in the classical A and B forms, but a dinucleotide pair. This gives rise to a modified D form for poly (dA-dT) (and poly (dI-dC), which is isomorphous) with a 4:1 (8:2) helix and an analogous 5:1 (10:2) B form for poly (dG-dC). In these wrinkled helices the RpY step is the $O_{3'}$ P step (angle ζ in Fig. 14) in the trans conformation, whereas in the YpR step this angles is in the usual gauche form. Finally, a pleiomeric form of poly (dA-dT) was recently described (115). This unusual form has a hexanucleotide as repeating unit, with different conformations between the different ApT and TpA steps.

Heteromerous (or heteronomous) helices are formed by hybrid duplexes, which contain ribopolymers in one strand and deoxypolymers in the other.

base (Hình 31b).

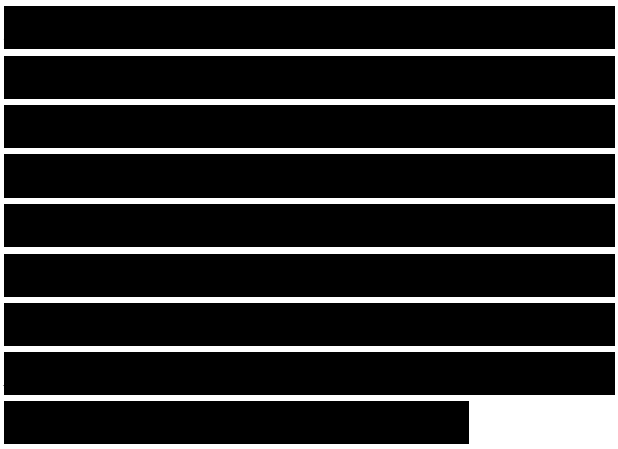
[redacted]

In poly (rA) poly (dT) each strand maintained its conformational features, ie, 3'-endo for the RNA strand and 2' – endo for the DNA strand (155). Recent work (119) has confirmed this for poly (dA) poly (rU) and poly (dI) poly (dC). A heteromerous struc-ture was also found (110) in poly (dA) poly (dT), an all – DNA double helix, where poly (dA) shower the unusual 3'- endo conformation of the sugar, characteristic of the A family of polynucleotide secondary structure. All these structure give rise to double helices where the radius of the A part is larger than that of the B part. Such structure are reminiscent of those suggested by others (134).

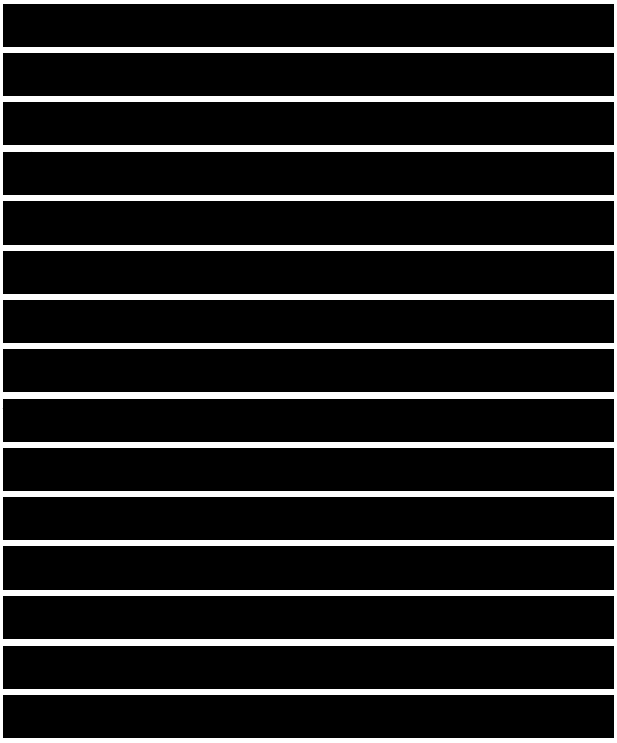
The new x-ray fiber structure account well for the unusual solution data observed by cd (27,129-132) and Raman spectroscopy (156), but not necessarily by nmr (157). Nmr relaxation and 2 – D NOESY (153) did not show any difference in the strands of poly (dA) poly (dT). Similary, equivalent strands were found (158) in poly (rA) poly (dT) using NOE measurements. It was shown that several DNA duplexes have fast, large – amplitude local internal motions (± 20-25^{o}) in solution and that amplitudes of bases and sugars are similar. It appears

that the dynamics of the polynucleotide have an averaging effect on the structure, which when "frozen" ina fiber is maintained in a unique form. The multitude of fiber diffraction structure, however, points to the great variability of conformations of the polynucleoticde duplexes.

Fig. *30. (a) Molecular models of A and B forms for the dodecamer sequenced (CGCGGATCCGCG) using the coordinates of Ref. 154. Courtesy of J. Sherfils.(b) Schematic presentation of A – and B – DNA; base paris q and 11 in B- DNA and base paris 1 and 12in A-DNA coincide exactly.*



There are strong indications that hydration may be the driving force of the B-to-A transition. Continuous changes were observed (159) from B-to-A-DNA in x-ray fiber diffraction patterns of samples from ethanolic solutions, whereas A-T-rich DNAs undergo the B-to-A transition only with a difficulty (160, 161). Recent work (162, 163) on oligonucleotide suggests that A-T-rich regions are stabilized by a water spine. On the other hand, only poly (dG-dC) forms the sZ form, poly (dA-dT) dose not, except under torsional strain (164). Substitution of G by I also abolishes the Z form. The base composition as well as sequence thus play an important role in determining



structures of synthetic DNA.

The data in Table 3 have been obtained from fiber x-ray diffraction data (Fig. 29). Other techniques have been used to study DNA conformation in solution. Cd (165) established that DNA changed its conformation from B-to-A-DNA in ethanolic solutions (Fig. 32a). X-ray scattering showed that DNA could assume different forms, depending on its base composition (160). Using ir dichroism (161), the B-to-A transition in films of DNA was characterized as a function of the G-C content; furthermore, it demonstrated the absence of the A form in A-T-rich DNAs (Fig. 32b).

Fig.32. *(a) B-A transition followed by cd (165). Insert shows mole fraction A as a function of percentage of ethanol. Numbers on curves are percent ethanol. Courtesy of Academic Press, London. (b) B-A phase diagram observed in fibers (by x-ray diffraction) and films (by ir cd). Data from Ref. 161. Courtesy of MacMillan Ltd, London.*

On the other hand RNA assumes solely the A form or its derivatives. It always has the 3' – endo sugar pucker

conformation and is right-handed. A recent paper (166), however, reported the formation of a left – handed helix by poly (rG-rC). The DNA-RNA hybrids usually assume the A-form geometry in both strands (118,120), although recent data (119,155) have demonstrated the existence of mixed A/B heteromerous double helixes. This structural conservatism of RNA as opposed to the polymorphism of DNA isrelated to the difficult with which ribosides transit from the 3'- endo to the 2' – endo conformation. The considerably higher energy barrier between the two conformations in ribose as compared to the small energy involved in the analogous transition in deoxyribose is accentuated in a polymeric structure. Every movement and change in a torsion angle is propagated along the polynucleotide chain through the seven conformational angles (Fig.14).

Z-DNA. When increasing the salt concentration in a solution of poly (dG-dC) to 4 M, a dramatic inversion of the cd spectrum was observed (167) (Fig.33a). This transition was cooperative with slow kinetics and an activation energy on the order of 100 kJ/mol (24 kcal/mol). The low salt form was called R, and the high salt form L; a right-left-hand transition in the sense of the helix was proposed. Other agents were also found to convert poly (dG-dC) from the R to the L form, including alcohols at 60-65% (168,169), di- and

trivalent cations, as well as amine complexes of cobalt, platinum (Ⅱ) zinc, and copper (170-173).

Fig. 33. (a) Cd spectra (173) and (b) ir (B) and Raman (A) spectra of poly (dG-dC) in the B and Z form. Courtesy of Prof. E. Taillandier and Dr. R. Taboury.

It took several years before a small fragment, $(dC\text{-}dG)_3$, was synthesized and studied by x-ray crystallography; it was show to form a left-handed helix, called Z-DNA (174). Reinterpretation of older x-ray fiber diffraction data (108) clearly shower that such left- handed helixes could also be formed by poly (dG-dC) and that the L form and Z form were identical (116). Similar repeating sequences, such as poly (dA-dC0 poly (dG-dT) also form left – handed Z-DNA (116).

In addition to the left- handedness of the helix, Z-DNA exhibits several striking features (175). The repeating unit of the double helix is not a mono- nucleotide pair, but a pair of dinucleotides, ie, the dyad is between the bases, not in the plane of the base. The orientation of the bases around the glycosidic linkage is anti for the deoxycytidine residue, but syn for the deoxyguanosine residue (Fig.12). the sugar puckering is

alternatingly 2' –endo in deoxycytidine, but 3' – endo for deoxyguanosine. These changes also affect the ir and Raman spectra (Fig. 33b) of poly (dG-dC) (176,177). The sugar- phosphate backbone of the double helix is not continuous as in the right – handed A and B forms of DNA, but follow a zigzag pattern to accommodate the unusual glycosidic linkages and puckers with the two sugars facing each other. The dCpdG sequence is thus not equivalent to dGpdC. This nonequivalence of the phosphates gives rise to two distinct phosphorus resonances in Z-DNA. Although in A – and B-DNA two distinct grooves are observed, only one can be distinguished in Z-DNA, which is much deeper. The second is a broad outside crest, caused by the flip- over of the base pairs outside the helix.

Various factors influence the formation of Z-DNA. In Z- DNA the phosphate-phosphate distance across the groove is only 0.77nm, compared with 1.17 nm in B-DNA. This large electrostatic repulsion makes Z-DNA intrinsically unstable. The original finding that high salt concentrations stabilize Z-DNA (167) is not surprising. Topological stress in closed supercoiled circular DNAs, which contain alternating purine-pyrimidine sequences, also

induces left- handed DNA (175).

Chemical modification that stabilize Z-DNA have been studied extensively. Bromination of deoxyguanosine yields dbr^8Guo, which is confined to the syn conformation (178). This reaction has been used (179) to brominates poly (dG-dC) in 4M NaCl. After the reaction, the salt was removed by dialysis, but the polymer stayed in the Z form. This polymer was used to elicit antibodies against Z-DNA, which proved extremely useful in localizing left-handed DNA structure in natural DNAs (175).

Bromination or methylation of deoxycytidine on position 5 also stabilizes Z-DNA. Thus poly (dm^5C-dG) undergoes the salt – induced B-Z transition already at 0.6M NaCl (170). This observation is of considerable interest since cytidine methylation of CpG sequences is one of the most abundant natural modifications of DNA and appears to play an important role in the control of gene activation in prokaryotes and eukaryotes (180).

The crystal structure of d $(m^5C\text{-}G)_3$ (181) futher indicates that the two methyl groups of opposite strands are on top of each other on the large groove

crest and shield the access of water molecules to the hilex. It thus also favors Z-DNA formation in alcohols. A futher chemical modification (182-184) is the reaction with N-acetoxy	[redacted]

Aminofluorene (AFF), a potent carcinogenic drug. This reaction, like bromination forms covalent bond with deoxyguanosine on position C8 and thus induces the synconformatinon. Alkylation on position N7of dGuo also induces Z-AND (184). Since most alkylating agents are carcinogenic, there has been much speculation on the role of A-AND in cancer (171, 185)	[redacted]
Supercoiled DNA. The studies reported so far have dealt with synthetic polynucleotides, which by definition are linear. The situation is quite different in natural DNAs, which are circular are supercoiled with very few exception.	[redacted]
Supercoiled results when the two strands of the double helix of a closed circular DNA are underwound (negative supercoiling) of overwound (positive supercoiling). In both case an unfavorable free-energy change relative to the relaxed closed circular DNA exists. Any reaction or process that reduces the degree of supercoiling takes place more easily in the supercoiled than in the relaxed state, eg, intercalation of dyes. The two strands of a closed circular doublehelical DNA in the relaxed state are linking number α. In the relaxed state it measures the number of helical turns α0 and is directly proportional to	[redacted]

the number of base pairs. The actual linking number is, however, different, because most circular DNAs are supercoiled; thus the topogical linkage of the double helix is difined as (186).

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Or as specific linkage

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All natural circular DNAs are negatively under wound with …. And….

By appropriate manipulations (187), populations of supercoiled double-heli-cal DNA molecules that have different topological linking numbers (topoisomer) can be produced. The topoisomers have different hydrodynamic properties and can be separated by one-and two-dimensional gel electrophoresis. Negative supercoiling has been investigated , extensively in recent years because of its ubiquitousness. Specific enzymes, tobosomerases, control the degree of supercoiling in the cell (187), negative supercoiling has been found to be the most important stabilizing factor of Z-DNA in naturally occurring DNAs (187-190).

Oligonucleotides

Chemical Synthesis Of Oligonucleotides. The chemical synthesis of oligonucleotides has been dramatically in the last decade (191-194), primarily because of the large demand of oligonucleotides for biological applications in genetic engineering. The improvement of syntheses and yields has also permitted the study of oligonucleotides by physical methods, principally x-ray crystallography and nmr.

In order to control the synthesis of an oligonucleotide, certain reactive groups have to be protected by easily and reproducibly cleavable groups have to be protected by easily and reproducibly cleavable groups. The preparation

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Fig. 34.Oligonucleotide synthesis by (a) the phosphotriester method and (b) the phosphite-triester method. DMT: 4,4'-dimethoxytrityl; Z: 3'-protecting group or solid support; R: Clor amine (morpholyl, dimethylamino, diisopropyl-amino, etc); Bt, B2, Nl N2: protected bases (193).

of the reagents is thus the most important and difficult part of the synthesis (195-198). Most of the reagents are now commercially available at fairly reasonable prices. Automated systems have been developed and are also on the market. Oligonucleotides up to 50 and 70 nucleotides long have been synthesized. It is now possible, even for an inexperienced investigator, to prepare milligrams of oligonucleotides 15-20 nucleotides long on a DNA synthesizer in two days.

Oligonucleotides can be synthesized chemically in solution or on solid support. The latter method has increased the yields and speed of the coupling reactions. The quantities that can be produced are, however, much smaller. It is thus the method of choice for the synthesis of small amounts of long-chain oligonucleotides (up to 70 nucleotides) as they are used for biological studies and genetic engineering. For the preparation of

large amounts of shorter-chain oligonucleotides (<20 nucleotides) for physical studies, the solution method is generally preferred, but requires considerably more time and material.

The two principal methods are the phosphotriester method (Fig. 34a) and the phosphite-triester method (Fig. 34b). Both methods have advantages and inconveniences and follow similar paths: first, attachment of the 3' terminal

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nucleotide to the solid support (or protection of the 3'0H group in the solution method); second, coupling with the following nucleotide; and third, capping and removal of the unreacted nucleotide. This process is repeated with the coupling and capping steps of the next nucleotide until the entire chain has been built up. Finally, there is deprotection in several steps: acid hydrolysis removes the 5'-protecting group (generally 4,4'-dimethoxytrityl); alkaline hydrolysis removes the groups that protect the amino groups of the bases, usually benzoyl groups

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for adenine and cytosine and isobutyryl groups for guanine. The removal of the isobutyryl groups is essential for physicochemical studies and is only possible in very concentrated ammonia at 60°C under pressure. The oligonucleotides are finally purified by hplc or gel electrophoresis.	[redacted]
The chemical synthesis of oligoribonucleotides is considerably more difficult. Protecting the 2'OH group is not very selective (197) and thus 3’-5' and 2’-5' chains can be formed; furthermore, oligoribonucleotides are unstable to alkali, and therefore most of the protecting groups used in DNA synthesis cannot be used.	[redacted]
Single-crystal X-ray Studies. Molecular Conformation of A-, B-.and Z-DNA. The development of synthetic methods for the preparation of oligonucleotides has permitted for the first time the synthesis of sizeable amounts (tens of milligrams) of DNA. Although the first synthetic DNA pApTpApT was not a regular double helix (199), x-ray analysis of single	[redacted]

crystals has since permitted the characterization of both the A and B families of DNA to atomic resolution and the discovery of left-handed Z-DNA. These studies, besides confirming most of the results obtained from x-ray fiber diffraction studies, revealed structural details totally inaccessible by fiber work, such as variations in helix-turn angles between successive bases. The resolution of tightly bound water molecules has permitted investigation of DNA hydration. These data have suggested mechanisms by which individual base pairs could be recognized by enzymes or drugs.

The essential features of single-crystal studies published between 1980 and 1985 are given in Table 5. It is striking how well most of these results agree with those in Table 3 obtained from fiber work. A-DNA (Fig. 35a) is a rather broad right-handed helix with the bases inclined; the large groove is very deep, whereas the small groove is very shallow. DNA dạng B (Fig. 35b) is slimmer, and the base pairs are essentially perpendicular to the helix axis; the two grooves are both distinct; the major groove is considerably deeper. Z-DNA (Fig. 35c) is still more

stretched out and left-handed, and the bases are only slightly inclined (in the sense opposite to that in A-DNA); the major groove is flattened to a crest, and the minor groove is the deeper.

Table 5. Structural Parameters of DNAs from Single-Crystal X-ray Structure

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Fig. 35.Molecular models of B-, A-, and Z-DNA from single – crystal studies. Stereo drawings using coordinated from Refs. 209, 201, and 174, respectively.Courtesy of Dr.E.Westhof.

Although these structural features are essentially similar to those from fiber work, there are some very important differences. Whereas A- and Z-DNA appear to be homogeneous in their structures, this is not in DNA dạng B. This essentially confirms the rigidity of the A form of DNA and the polymorphism of DNA dạng B . The difference are, however, considerably larger than expected.

In the best refined structure of DNA dạng B, the dodecamerd(CGCGAATTCGCG), the standard deviations are high and indicate the large difference between individual base (201, 202). Thus, although the mean turn angle between bases is 35.9o (ie, 10 base pairs per turn), corresponding exactly to the 36o obtained from fiber diffraction data, the standard deviation SD is 4.2o. The observed variations between individual bases range from 27.4o (ie, 13,1 base pairs per turn) to 41.9o (ie, 8.6 base pairs per turn). These large variations were totally unexpected.

Interestingly enough, these differences between the base pairs are not random, but follow precise patterns, ie, they are characteristic for each dinucleotide sequence. Kinectic studies on the cleavage of d(CGCGAATTCGCG) by DNAse I have shown that the rate constants of cleavage at each internucleotide linkage are different and are derectly related to the helux conformation at the step cleaved (203). This argues strongly for essential correctness of the helical structure derived from x-ray work and its similarity or identity with that found in solution. These conclusions were also confirmed by nmr. These data confirm

the strong heterogeneity and the polymorphism of DNA dạng B.

Structural Details. The large differences in helix twist between successive base pairs in the dodecamer d(CGCGAATTCGCG) have given rise to a series of further observations and considerations (162,163,204) on the effect of sequence on DNA structure. These details have changed the longstanding view on the uniformity of the DNA B helix. In Figure 36 the distortions that an individual base pair can undergo within the helix are shown. The base pairs are not flat,

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Fig. 36. Conformational properties of individual base pair. Propeller twist : dihedral angle between individual base planes along the axis of the base pair. Helix twist t: deviation from the standard orientation (helix turn angle) of the base pair relative to its neighbors. Tilt

(Table 3): orientation of the plane of the base pair relative to the helix axis along the pseudodyad axis. Roll : orientation of the plane of the base pair relative to the helix axis along the normal to the pseudodyad axis. All angle are measured positive in the clockwise derection. Slide, longitudinal and lateral (also called shear) are the displacements of the base pair in the plance away from the helix axis.

but have a "propeller twist" between the two bases of 10-20o (Figs. 36 and 37). This propeller twist enables a more efficient overlap of neighboring bases in a given strand. In homopolymer duplexes the propeller twist is a strong stabilizing principle in nucleic acid structure, even with the bases of the opposite strand twisted in the opposite sense.

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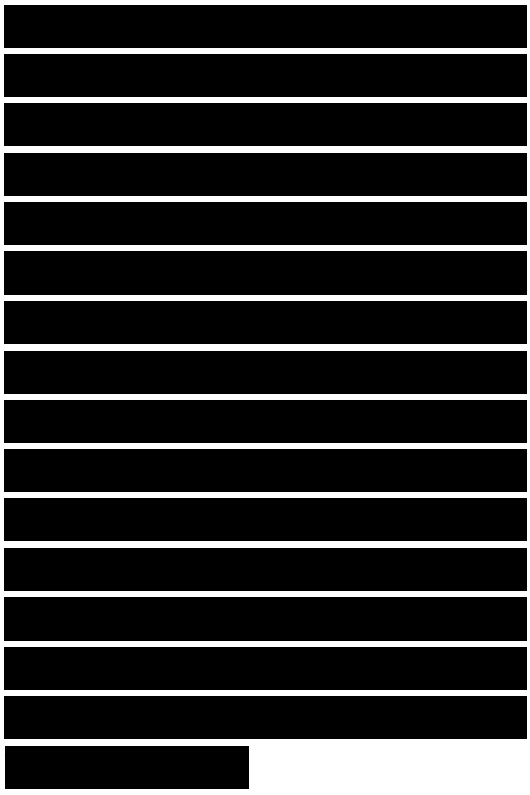
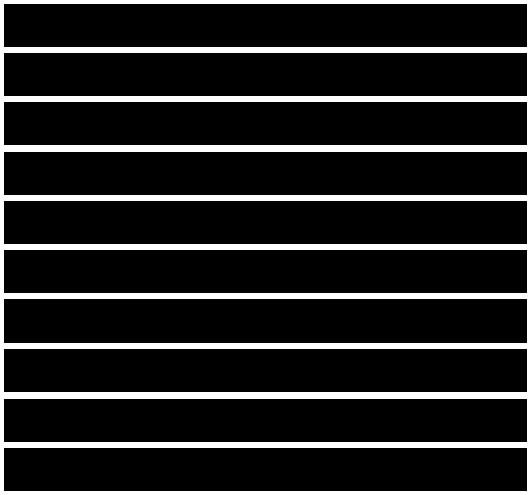
Fig 37. Effect of propeller twist in different base-base steps. (a) Purine-pyrimidine step: clash in the major groove (arrow head). (b) Purine-purine (or pyrimidine – pyrimidine)

step: no clash. (c) Pyrimidine – purine step: clash in the minor groove (arrow head) (200). Courtesy of Academic Press, London.

In DNA sequences, however, purin and pyrimidine bases may or may not, alternate along the strand. Since purin bases are larger than the pyrimidines, they enter in contact if they are in the opposite strand (double arrows in fig. 37). In a pyrimidine- purine step (Y-R, in the 5'-3' direction of the chain; Fig. 37c) this steric clash occurs in the minor groove, whereas in an R-Y step (Fig. 37a) it happens in the major groove. No such clash occurs in a R-R or Y-Y step (Fig. 37b).



The principles of elastic structural mechanics of beams have been applied to nucleic acid structure (204). Using the published x-ray data from studies of d(CGCGAATTCGCG) (201, 202) four basic rules, which consist of specific maneuvers to relieve the stiric hindrance between the successive purine bases, were proposed. The first two rules imply rotations in the plane of the base pair; the last two rules imply movements in the plane of the



base pair normal to the helix axis.

1. The propeller twist is reduced or suppressed.
2. The base pairs are rolled as a rigid unit along the C1'-C1' axis (θR; Fig. 36).
3. The base pairs are shifted along this virtual bond to the purine side (Fig. 36, slide).

4. The base pairs are rotated along the helix axis to relieve purine- purine contacts on opposite chains (local helix twist; Fig. 36).

Finally, the tilt of the base pair (Fig.36) relative to the helix axis can be adjusted.

The variations in local conformations of the nucleotides (bases, sugars, and phosphates) can essentially be accounted for by these maneuvers. In particular it was observed that the torsional angles δ (C5'-C4'-C3'-O3', which define the sugar conformation, Fig. 14) of the sugars attached to a given base pair were strongly anticorrelated, ie, if δ of the sugar of the purine nucleoside was large, that

of the pyrimidine nucleoside was small, and vice versa. Shift (rule 3) of a base pair relative to its two neighbors cause precisely this change in the phosphate-sugar backbone and thus in the sugar conformation.

These principles were further developed (162,205), and four simple sum functions were introduced to account for the four earlier rules (204). ∑1 measures the perturbation of the local helix twist angle t and is the sum of three contributions:

1. In a step x-R-Y-x the main-groove clash (Fig. 37a) is presented by the sequence +1,-2, +1, which emphasizes the fact that a given decrease in helix twist angle (set at -2) is reflected on both sides by half this value in the opposite direction.

2. In a step x-Y-R-x the clash in the minor groove is twice as severe (Fig. 37c). Therefore values of +2, -4, +2 are assigned to the step.

3. In x-R-R-x or x-Y-Y-x step no steric hindrance occurs between

purines and therefore no contribution is added.

The various contributions along the double helix are summed and plotted in Figure 38a.

The remarkable correlation between the sum function ∑1 and the observed helical twist angles t indicates the correctness of this approach; one "unit" of ∑1 corresponds to 2.1o of helix twist, with ∑1 at 35.6o in excellent agreement with the 36o from fiber diffraction data (Table 3).

Similar sum functions have been applied to the three other maneuvers: ∑2 sums the contributions of the roll angles (θR) between successive base pairs; ∑3 sums the contributions of the differences in sugar torsion angles δ ; and ∑4 sums

Fig.38. Sum functions ∑1 and ∑2 (200). (a) of the B-form dodecamer (d(CGCGAATTCGCG) and (b) of the A-form octamer d(GGTATACC). ●—●, observed twist (t) or tilt (θR angles, respectively; ▲---▲, computed ∑1 or ∑2values, respectively. Courtesy of Academic Press, London.

That of propeller twist θp.n The

various contributions can be summarized as follows:

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As can be seen in Figure 38, the first of the sum functions accounts remarkably well of the observed effects both in B and A-DNA. The last two, ∑3 and ∑4 (not shown), appear to be followed correctly only in DNA dạng B. In A-DNA stronger steric constraints seem to be the cause for these deviations.

In addressing the problem of the B-A transition further (205), an additional parameter, which describes the translation of the base pair in its plane, was observed which suggests that certain steps, in particular, Y-R steps (ie, C-G, C-A, T-G, T-A), are "bistable" or can assume either the A- or B- form geometry, because of highly favorable base overlap in both conformations, using twist, rolls, and slide of the base pairs; other sequences, in particular, A-A or T-T, are more favorable in the B form.

Clearly, these rules still have rather speculative character, but have helped considerably in the understanding of

the mobility and dynamics of DNA structure. With new data both from x-ray and nmr appearing rapidly, the description of helix geometry will become clearer.	[redacted]
Interestingly enough, the first helical structure solved were of the A-RNA type. They were formed by the simple dinucleoside phosphates GpC and ApU (206). Deoxyoligonucleotides require at least a tertramer to crystallize (199,201,202,207,208).	[redacted]
Hydration of DNA. The existence of two forms of DNA as a function of relative humidity was revealed early by x-ray fiber diffraction (14,15,106,107,109,154). The question of the role of hydration in DNA structure and stabilization has occupied researchers ever since.	[redacted]
The crystal structure of oligonucleotides reveals several important features of hydration and the differences between A and DNA dạng B. In both forms the phosphate groups are highly hydrated, and 2 to 3 water molecules per phosphate can be localized. In the A-DNAs, such as d(CCGG) (208) or d(GGTATACC)	[redacted]

(209) (Fig.39a), the water molecules produce a cross- linking network between the phosphate groups of opposite chains across the large groove, which itself is lined by water molecules bound to N and O atoms. In the very shallow small groove, only an irregular array of solvent molecules has been found do far.

In DNA dạng B, exemplified by d(CGCGAATTCGCG), there is a striking difference: a precise spine of water molecules runs across the small groove in the central AATT block of the dodecamer (Fig.39b). Water molecules bridge the N3 atoms of adenine with the O2 – keto group of thymine (162,163). These water molecules are themselves bridged by another molecules, giving rise to this spine. The presence of 2-amino groups in the guanine base blocks its continuation. It appears that the water spine of the minor groove contributes considerably to the stabilization of A-T- rich regions of DNA dạng B by water molecules. These observations suggest several explanations of the x-ray data. The breakage of the water spine appears to be the crucial step in the B-A transition (160-163). The difficulty of a B-A transition in A-T- or I-C containing polynucleotides can

be explained by the stability of the tightly bound water structure in the small groove, which maintains the B form to very low values if relative humidity. Similarly, it can be argued that among alternating polynucleotides only poly (dG-dC) can form the left-handed Z form because of its lack of stabilizing water structure. Poly (dA-dT) is totally refractive to the B-Z transition because it is "trapped" in the B form, whereas poly (dA-dC) poly (dG-dT) assumes the Z form only under very special circumstances (210).

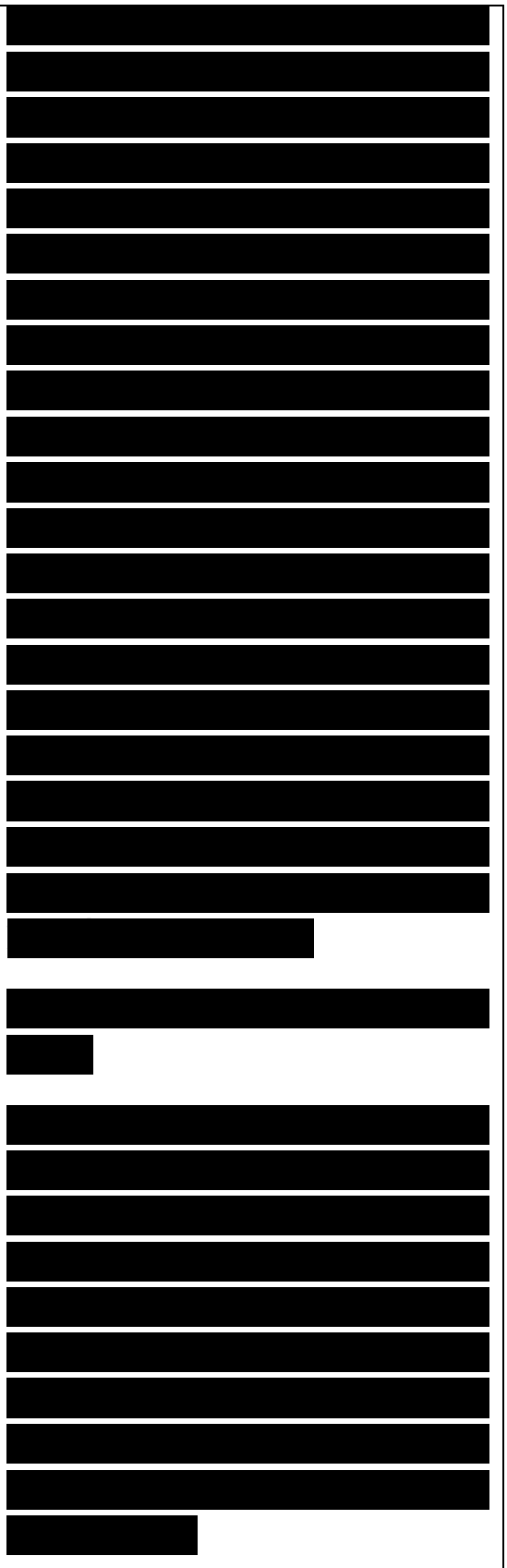

Fig.39. (a) Hydration network across the large groove in A-DNA (209). Courtesy of Adenine Press, Guilderland, New York. (b) Hydration spine in the minor groove of DNA dạng B (162,163). Courtesy of Academic Press, London. ●, phosphorus atoms; •, water molecules.

NmrStudies. Single- crystal x-ray studies have brought to light a wealth of structural information inaccessible by other methods. This has permitted the formulation of numerous new concept and opened the possibility of solving more complex problems, such as protein-nucleic acid interactions (211,212).

The information from x-ray work, however, is static and gives no indication of the dynamics of nucleic acid structure. In the case of an equilibrium between two states, the crystal freezes out one of the two states. It is rare that the same compound can be studied in two different conformations by x-ray techniques. In the case of nucleosides and nucleotides two (or more) asymmetric units in the unit cell have been reported. But it is improbable that oligonucleotides could be observed in two different states in the same crystal by x-ray methods. Or, the principle of operator- repressor interactions implies more than one state (213,214).

Clearly, the technique to study the dynamics of nucleic acids is high resolution nmr. The advent of high

field superconducting magnets, Fourier transform techniques, and new computer technology combined with the availability of sizeable amounts of oligonucleotides hs led to innumerable nmr studies on the structure and dynamics of nucleic acids.

Early studies were performed on small polynucleotides and DNA fragments of ca 200 nucleotides in length obtained by sonication (213). These studies were a direct extension of work on t-RNA (214,215) and permitted the first direct observation of the exchangeable imino protons of dGuo and Thd (216,217). The development of new pulse techniques (217-219) opened the possibility of measuring selectively the relaxation properties of A-T (around 14ppm) and G-C base pairs (around 13ppm). It was thus demonstrated (216) that the opening rates of base pairs in DNA and polynucleotides can be measured by selective pulse techniques. The demonstration that each base pair opened separately and independently of its neighbors permitted the determination of the activation energies of A-T and G-C base pairs in DNA as ca 71kJ/mol (17 kcal/mol) and 83kJ/mol (20 kcal/mol), respectively (Fig.40).

The chemical shifts of imino proton resonances strongly depend on the environment and thus in the structure of the nucleic acid studied. It is therefore not surprising that the chemical shifts in the B form are different from those in the A form (220). Junctions between B and A forms have been investigated in a polynucleotide oligonucleotide hybrid poly (dG) (rC11dC16) (221).

The most interesting and successful methods are the use of NOE measurements, the development of two-dimensional (2-D) nmr methods, and the use of H2O as solvent to study the dynamics of exchangeable protons. The first is by far the most interesting method, both in the standard 1-D (222-225) as well as in the 2-D technique (NOESY) (226-234). The sensitivity of NOE measurements is due to their 1/ (rij)6 relationship, ie, any measurable NOE indicates a distance between the irradiated and the enhanced proton of less than 0.5nm.

Fig.40. Temperature dependence of

longitudinal relaxation rates of imino protons of DNAs of various chain lengths (indicated). Activation energies of opening rates of DNAs and polynucletides can be computed from the slope. O, 12 base pairs; ●, 43 base pairs; ▲, 69 base pairs (216).

The environment of the protons in a nucleic acid helix (Fig. 41) permits the assignment of the structure of a DNA by four strategies:

1. Irradiation of the aromatic protons (H8 of purines and H6 of pyrimidines) gives rise to NOEs with aromatic proton of certain base above and below, depending on the sequence (double arrows in Fig. 41a). These through- space dipolar- couple interactions are very precise. Irradiation of H8 of a purine or H6 of a pyrimidine gives rise to a NOE to the H5 of cytudine or thymidine methyl group in the 3' direction (and vice versa), but not to the H8 of a purine. No NOEs are observed between H8 of a purine (or H6 of a pyrimidine) and H8 or H6 in either direction; the distances between these protons are over 0.5 nm.

2. Upon irradiation of the aromatic proton, NOEs are simultaneously observed on the H1', H2' and H2'', protons of the sugar attached to the base, but also to those of the sugar of the nucleoside attached in the 5' direction (Fig. 41b and c). Depending on the form of the helix (B or A), these internucleotide distance are very different. For instance the H8 (or H6) to H1' distance is ca 0.29 nm in DNA dạng B (Fig.41B), but ca 0.40 nm in A-DNA (Fig. 41c); in addition the distances between the aromatic proton and H2' decreases from ca 0.40 nm in DNA dạng B to 0.15 nm in A-DNA. These are extremely precise sensor of helix geometry. Evidently, the reversed NOEs, ie, from sugar to aromatic protons, can in principle be measured. The overlap between these aliphatic protons, however, always causes irradiation of several resonances in NOE experiments, thus complicating the interpretation of NOE spectra.

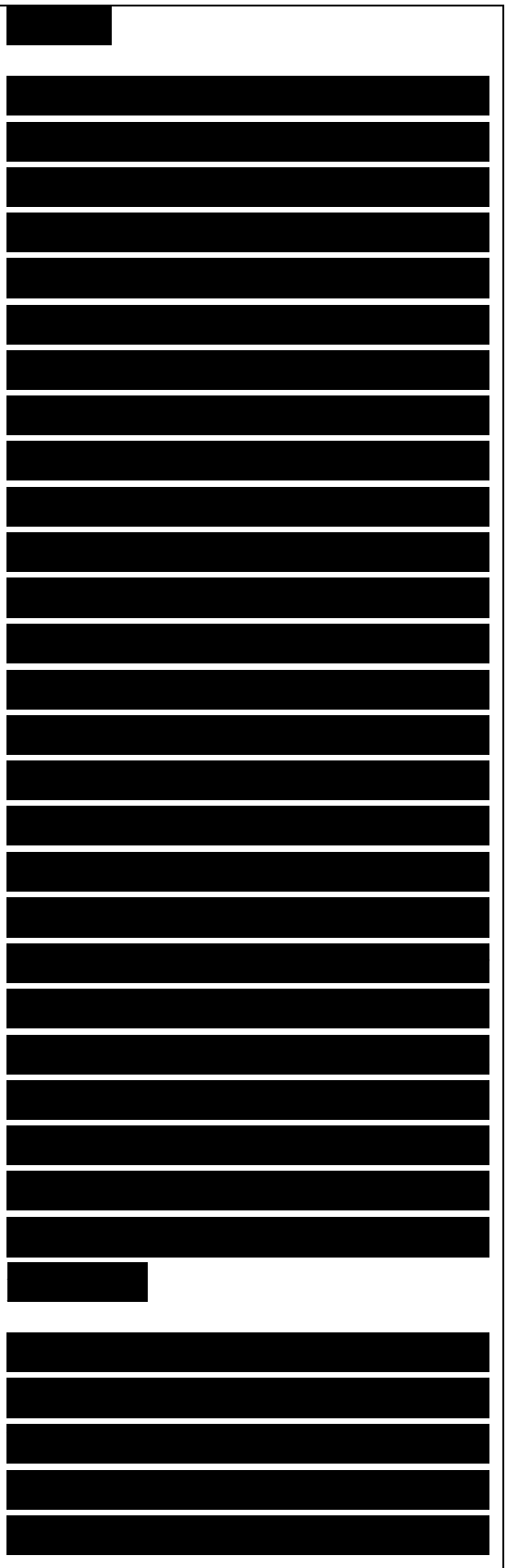

Fig.41. (a) Part of a DNA dạng B double helix with selective interproton NOEs indicated. ●, aromatic protons and (◄---►), NOEs with neighboring bases; ◊, imino protons and (◄–•–•–►), NOEs with neighboring bases; ◄••••►NOE between TH3

(imino proton) and AH2 (aromatic proton). (b) Intra – and interproton distances (in nanometers) between H6 and the sugar protons in the B form. (c) Intra- and interproton distances (in nanometers) between H6 and the sugar protons in the A form. Analogous distances between H8 of purines and the sugar protons are only ± 0.02 nm different. Adapted from Ref. 226. Courtesy of Adenine Press, Guilderland, New York.	[redacted]
3.Irradiation of the exchangeable imino protons in H2O solutions again permit specific assignments of neighboring bases. Since the imino protons are virtually on the helix axis in DNA dạng B (Fig. 29a) they are precisely 0.34 nm apart. The chemical shift differences between A-T and G-C imino protons thus permit the precise assignment of the sequence from the NOEs between them. An additional NOE can be observed between the thymine imino proton and H2 of the adenine with which it is paired. This permits the unequivocal assignment of the anti conformation of these base pairs. A syn conformation would yield an NOE on H8.	[redacted]

	[redacted]
4. Finally, recent advances permit the assignment of the amino protons from NOE measurements (228), which are in the 6-9 ppm spectral region. These assignments are clearly of great importance since it is these amino protons that are perturbed on interactions with ligands, such as drugs or proteins.	[redacted]
Two- dimensional (2-D) nmr techniques have been developed (229-231) for the sequential assignment of protein resonances. Resent applications to nucleic acids have facilitated the assignment of resonances and structures of oliginucleotides in a single experiment. The two preferred methods are NOESY (2-D NOE) and COSY (2-D autocorrelated spectroscopy). The first measures through -space (dipolar-coupled) connectivities, the latter through- bond (scalar- coupled) comectivities. Applications of NOESY to the study of nucleic acids have been extensively used in recent years (226,227,232-234) and have permitted assignment of most of the resonances and determination of the conformational features of many oligonucleotides.	[redacted]

Localization of the 5’ terminal base permits sequential assignment using the criteria discussed above (Fig. 41). Figure 42 shows an enlarged part of a NOESY experiment of the hexanucleotide d(GGATCC) in D2O. The connectivities between the aromatic and anomeric protons are indicated; the whole sequence can be followed according to the principles evoked above. Fig. 42. NOESY spectrum of the aromatic vs anomeric region of the hexanucleotide d(GGATCC). Courtesy of Dr.G.V. Faxakerley and Dr.E. Quignard.	[redacted] Sự cục bộ hóa các base đầu cuối 5 'cho phép ấn định tuần tự bằng cách sử dụng các tiêu chí được thảo luận ở trên (Hình 41).Hình 42 biểu diễn một phần mở rộng của một thí nghiệm NOESY d hexanucleotide (GGATCC) trong D2O. Các kết nối giữa các proton thơm và anomeric được chỉ ra, toàn bộ chuỗi có thể được suy ra theo nguyên tắc đã đề cập ở trên. Hình 42. Phổ NOESY của khu vực thơm (aromatic) và anomeric của d hexanucleotide (GGATCC). Được sự cho phép của Tiến sĩ GV Faxakerley và Tiến sĩ E. Quignard.
