PHYSICO-CHEMICAL PROPERTIES OF NUCLEIC ACIDS

Volume 3

edited by Jules Duchesne

Physico-chemical Properties of Nucleic Acids

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VOLUME 3: *Intra- and Intermolecular Interactions, Radiation Effects in DNA Cells, and Repair Mechanisms*

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Preface

Progress in the application of physics and chemistry to biology has been particularly important in a number of areas, and this book brings together recent work in these fields.

Nearly all the topics have been approached in the spirit of molecular biology and biophysics, where recent advances are far from having exhausted their impact.

Though the book has been subdivided into three parts for practical reasons, the unity of the whole work has been largely preserved and each volume is also an entity as it stands.

The first volume is mainly concerned with the intrinsic properties of nucleic acids, considered as macromolecules, and their components, and this study is completed by the analysis of different types of bindings or interaction mechanisms, including photodynamic and radiation effects, as well as fluorescence. In the second volume, the emphasis is put on structural studies and especially on conformational changes, using spectroscopic techniques as well as methods of thermodynamics and hydrodynamics. The stage of specific biological functions is attained in the last volume, with some considerations on repair mechanisms in relation to the general problem of evolution.

"The Physico-chemical Properties of Nucleic Acids" is intended to provide thought provoking material for research scientists, whether they are biologists, chemists or physicists. At the same time, it should be a source of information and reference for graduate students in these fields.

It is particularly hoped that these papers may help to stimulate the search for a better understanding of the correlation between structure and function. This understanding can only be founded on a detailed knowledge of the molecular properties of the basic substances.

Lastly, it is a great pleasure for the Editor to acknowledge the excellent cooperation of the publisher, who has been helpful in every way.

November 1972 JULES DUCHESNE

Foreword

The chemist, if he encounters a new substance, is accustomed to look first for its macroscopic properties in order to correlate them with underlying structural features. Such properties instantaneously suggest to him some picture of the molecule, and often it is only the material quality which stimulated his interest in particular substances.

If we think of nucleic acids, such a correlation does not usually come to our mind. In fact, most of us working on one or the other aspect of this fascinating molecule, have not even seen any pure crystalline material, not to speak of particular samples such as isolated gene material or single uniform *t*RNA batches, which actually are the objects of the most exciting studies in molecular biology.

Speaking of nucleic acids we usually do not associate with them any characteristic material property; we rather think of some abstract quality: information, instruction, translation, etc. We see before us sequences of letters, such as

$$\ldots \text{pApUpGpCpGpUpApUpApGpCpApUp} \ldots$$

and we think of a message. Of course, this is an exaggeration. Most of us—encountering any analysed sequence—would immediately start to play around with it and fold it up, e.g. as

$$\begin{array}{r} \ldots \text{pApUpGpC}^{\text{pG}}\text{pU} \\ \text{pA} \\ \ldots \text{pUpApCpG}_{\text{pA}}\text{pU} \end{array}$$

This shows that we actually associate with each letter also a particular physical interaction unique to this species of macromolecules.

It is this interaction, this exclusive way of complementary pairing of bases which is behind the abstract property of "code reading" or "information transfer", and it was the realisation of this quality which led Watson and Crick to their epochal discovery (quantitatively manifested in Wilkin's X-ray diffraction data).

How far our interpretations are guided by abstract reasoning became especially apparent in the deciphering of the genetic code. With 20 amino acids (plus some punctuation symbols) to be coded by the four bases (i.e. A, U (or T), G and C) it was "obvious" that the code had to be a triplet

code—as indeed has been confirmed by the work of Nirenberg, Matthaei, Khorana and Ochoa. However, this was "obvious" only by logical arguments: a doublet code could only provide 16, i.e. less than 20 codons, a quadruplet code would be uneconomical in providing too many, i.e. 256 codon units. But how could molecules be so intelligent as to accept a logical argument? The answer is: It is the physical behaviour of the nucleotide sequences which determines the optimal choice among the different possible associations and the one chosen appears to be in agreement with our rational logic, because it offers evolutionary advantages with respect to precision and speed of information transfer. Precision requires interactions of sufficient stability, thus it involves "stickiness" which limits the rate of information transfer. The codon–anticodon interaction therefore must be optimised to involve:

1. sufficient functional capacities (i.e. requiring more than twenty combinations)
2. distinctive recognition (requiring at least base triplet interactions) and
3. sufficiently low stickiness (keeping the codon-unit as small as possible).

What I wanted to say is that all abstract qualities of nucleic acids which we associate with their function to store, transfer, and process information are reflected by certain unique physico-chemical properties. It is not sufficient to have just macromolecular species resembling a sequence of different digits. The "digits" in addition must provide very specific physical interactions to cause the inherent property of self-instruction and code formation. This quality, unique to the nucleic acids (and their interactions with proteins) provides the capacity of self-organisation according to—or in agreement with—our abstract principles of purposefulness, usefulness and rationality.

In this situation it is highly desirable to use any available experimental tool to enhance our knowledge about the "Physico-chemical Properties of Nucleic Acids". The three volumes which appear under this title offer a large repertoire of studies. Not all of them may be equally relevant for an understanding of the characteristics of information processing, which also involves highly specific interactions of nucleic acids with proteins. Nevertheless, all these studies will finally contribute to our basic understanding of those properties, which are behind the structural features, specific interactions, and dynamic performances of these unique macromolecules. In our age of molecular biology it may seem to be somewhat fashionable to do research in the field of nucleic acids. However, there will be a long persisting interest in this field before our knowledge about the molecular details will have brought about a complete understanding of the sophisticated organisation of the genome of a highly developed cell.

Spiegelman once characterised the central role of nucleic acids by saying jokingly: "The evolution of life is a trick of nature to ensure a faster and better reproduction of the nucleic acids".

November 1972

MANFRED EIGEN

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Intra- and Intermolecular Interactions, Radiation Effects in DNA Cells, and Repair Mechanisms

CHAPTER 17

Renaturation of DNA in the Absence and in the Presence of Cu^{2+} Ions

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I. Introduction

The fact that DNA and certain polynucleotides exhibit a Watson and Crick double helix structure is now well known. This ordered structure is the native one in which the complementary base pairs Adenine-Thymine (AT) and Guanine-Cytosine (GC) are parallel to one another and perpendicular to the helix axis. Denaturation is the physical treatment which destroys this order. Renaturation is the reverse process, that is, the transformation from the disordered state to the ordered one.

II. Renaturation Kinetics of Polynucleotides in the Absence of Cupric Ions

As early as 1963, Marmur and his co-workers (Marmur *et al.*, 1963) completed a bibliographical study of renaturation of polynucleotides in the absence of ions such as Cu^{2+}. Wetmur and Davidson (1968) clearly summed up the known results to that time. However there has been little subsequent

advance of our knowledge in this field since this date. At the risk of repeating previously reported results we include here, for the convenience of the reader, all known results in a slightly modified form.

A. MATERIALS AND METHODS

From Table I it can be seen that various types of polynucleotides have been studied and that the most frequently used technique to measure the degree of renaturation α of the DNA as a function of time is u.v. spectrophotometry (hypochromicity). If α is obtained by measuring a property P, the optical density for instance, it is defined by the relation:

$$\alpha_P = \frac{P_1 - P}{P_1 - P_2}$$

where P is the value of the property P at time t and P_1 and P_2 are its value at the initial and final times respectively.

TABLE I

Authors	Polynucleotides	Techniques
P. D. Ross and J. M. Sturtevant (1960).	Poly (A + U)	u.v. spectrophotometry (Stopped-Flow)
J. Marmur and P. Doty (1961).	Bacterial DNA	u.v. spectrophotometry Transforming activity
L. F. Cavalieri, Th. Small and N. Sarkar (1962).	Bacterial DNA	u.v. spectrophotometry
R. B. Inman and R. L. Baldwin (1964).	Poly dI : $d\overline{BC}$	u.v. spectrophotometry
K. J. Thrower and A. R. Peacoke (1966).	Bacterial / Phage DNA	u.v. spectrophotometry
J. A. Subirana and P. Doty (1966).	Bacterial DNA	u.v. spectrophotometry
R. D. Blake and J. R. Fresco (1966).	Poly (A + U)	u.v. spectrophotometry
J. G. Wetmur and N. Davidson (1968).	Bacterial / Phage DNA	u.v. spectrophotometry
R. J. Britten and D. E. Kohne (1968).	Bacterial / Vertebrate DNA	Hydroxyapatite column
F. W. Studier (1969).	Phage DNA	Sedimentation velocity
J. G. Wetmur (1971).	Phage DNA	u.v. spectrophotometry

B. VARIABLES

The alteration of the value of some of the variables has the consequence of changing the rate of renaturation v.

A first attempt at enumeration of the variables has been done by Marmur and Doty (1961) with the purpose of defining the optimal conditions of the renaturation of DNA.

1. *Source of the DNA*

DNA samples from different sources can be distinguished by both base composition and by their complexity. On one hand the speed of renaturation v increases as a function of the percentage of GC base pairs in the DNA (Wetmur and Davidson, 1968), while on the other, all other things being equal, the speed of renaturation v of bacteriophage DNA is greater than that of bacterial DNA which in turn is greater than that of mammalian DNA. Britten and Kohne (1968) quantitatively established that the rate of renaturation decreased as a function of the length of the genome. In the same year Wetmur and Davidson (1968) reported an empirical relation between the rate constant k_2, the length of single strands of DNA, and the number of base pairs in non-repetitive sequences N:

$$k_2 = 3.10^5 . L^{0.5}/N$$

2. *Renaturation Temperature* T_2†

There are two distinguishable cases: that of synthetic polymers and that of DNA.

The formation of poly (A+U) (Ross and Sturtevant, 1960) and poly dI: $d\overline{BC}$ (Inman and Baldwin, 1964) is such that the speed of transformation increases as the temperature is reduced. If T_m is the melting temperature, the rate constant k is a positive linear function of $(T_m - T_2)$ (Ross and Sturtevant, 1960).

The renaturation rate of DNA passes through a maximum as the temperature decreases (Wetmur and Davidson, 1968; Marmur and Doty, 1961); the maximum occurring at around 25° below T_m, depending on the GC composition of the DNA.

Studier (1969) has shown that the degree of folding of single strands increases with temperature and that the renaturation rate of folded strands is less than that of the unfolded forms. In fact the effect of temperature on the rate v could be the result of the temperature effect on the folding of the single strands.

3. *Ionic Strength* μ *of the Solvent*

The degree of renaturation is optimal when the ionic strength, obtained by the addition of sodium ions, is greater than 0·4 M ($\mu > 0{\cdot}4$ M). All the investigators having studied the influence of ionic strength on the rate of renaturation (Wetmur and Davidson, 1968; Ross and Sturtevant, 1960; Inman and Baldwin, 1964; Thrower and Peacoke, 1966) report concordant results affirming that v increases with μ. Only Studier (1969) claims that v passes

† T_1 is used to denote the denaturation temperature.

through a maximum with increasing μ. This would seem to be connected to the combined effect of μ on the speed of transformation and the folding of the single strands.

4. *Solvent Viscosity* η

If the viscosity of the solvent is increased by the addition of sucrose (Subirana and Doty, 1966; Thrower and Peacoke, 1966; Wetmur and Davidson, 1968), glycerol or $NaClO_4$ the rate of renaturation decreases. In contrast, increasing η by the addition of Na polyacrylate or native T4 phage DNA results in an increase in v.

5. *pH of the Solvent*

The rate of renaturation increases with the concentration of DNA (Inman and Baldwin, 1964; Subirana and Doty, 1966).

6. *Molecular Weight–Length*

The degree of renaturation increases with molecular weight (Marmur and Doty, 1961).

Consider the equation previously cited (Wetmur and Davidson, 1968):

$$k_2 = 3.10^5 . L^{0.5}/N$$

7. *Degree of Folding of Single Stranded DNA*

Having prepared T7 phage single stranded DNA, Studier estimated the degree of folding by simultaneously measuring the optical density and the sedimentation velocity. It is possible to alter the folding of single strands by modifying the temperature, the ionic strength or the pH. Studier established that the rate of renaturation v is reduced as the degree of folding increases (Studier, 1969).

8. *Theoretical Studies*

The various theoretical treatments account for, in a satisfactory manner, the temperature effect and the length of the DNA (Ross and Sturtevant, 1960, 1962; Saunders and Ross, 1960; Wetmur and Davidson, 1968; Wetmur, 1971).

a. *Effect of temperature.* The simplest model which accounts for the variation of the rate of formation of poly (A+U) (Ross and Sturtevant, 1960) as a function of temperature is that of Saunders and Ross (1960). They consider that processes in polymers consist of a succession of identical steps carried out by each one of the monomer. Each step is supposed to be reversible and as a result there corresponds to each step two rate constants: one being k_f for the reaction under consideration while the other k_b is for the reverse reaction. Only one step is different from the others, that being the initiation

step for which the rate constant is k_i. The steady state approximation is assumed to be applicable. If the reaction is of the type:

$$A+B \rightarrow C_1 \rightarrow C_2 \ldots \rightarrow C_n$$

the rate of the reaction can be expressed by:

$$v = k_i [A] [B] \left(1 - \frac{k_b}{kf}\right)$$

Furthermore if it is assumed that the rate constants can be described by the Arrhenius equation, the expression for v becomes:

$$v = C \exp(-\Delta H^*/RT) [1 - D \exp(-\Delta H/RT)]$$

where C and D are constants, ΔH^* the activation energy and ΔH the enthalpy of formation of a step. At the melting temperature T_m, $v=0$ by definition and thus $[D \exp(-\Delta H/RT)]=1$. This latter expression is less than one when the reaction takes place. By adjusting the values of ΔH^* and ΔH the experimental results of Ross and Sturtevant (1960) for the formation of poly (A+U) can be accounted for.

The preceding model has been discussed and modified by Flory (1961), Ross and Sturtevant (1962), Kallenbach, *et al.* (1963), but the mode of calculation has remained unchanged. Finally Wetmur and Davidson (1968) introduced into this model the influence of random base sequences for DNA and in this manner explained the curve $v=f(T)$ observed for DNA (Marmur and Doty, 1961; Wetmur and Davidson, 1968).

b. *Effect of Chain Length.* The variation of the rate of renaturation as a function of chain length can be explained by an excluded volume theory. To do this, Wetmur (1971) has calculated the probability of two random coils approaching one another in such a manner that their centres of gravity are at a distance d and subsequently determined the rate of renaturation of the random coils situated at this distance. The rate of renaturation depends on the relative probability $P(d)$ of a configuration and also on the probability of overlapping of a given pair of segments.

This theory accounts very well for the second-order rate constant k_2 varying as $L^{0.5}$.

C. ANALYSIS OF THE KINETIC RESULTS

In order to process the kinetic curves obtained under different experimental conditions, different authors have tried to categorise the transformation studied. As is shown in Table II the order of the reaction is often poorly defined. It appears that the most recent studies (Britten and Kohne, 1968; Wetmur and Davidson, 1968) favour an assignment of second order.

TABLE II

Authors	Reaction order n
P. D. Ross and J. M. Sturtevant (1960).	$n=2$ at the beginning of the reaction $\rightarrow n=1$ near the end; the more μ diminishes, the more $n \rightarrow 1$.
L. F. Cavalieri, Th. Small and N. Sarkar (1962).	$n=1$ at $T=60°C$; second-order effect becoming more important when $T=70$–$80°C$
R. B. Inman and R. L. Baldwin (1964).	$n=2$.
K. J. Thrower and A. R. Peacoke (1966).	$n=2$ (first 3 hours) $\rightarrow n>2$ (longer times).
J. A. Subirana and P. Doty (1966).	$n=2$ with deviations; as increasing μ the deviations, relative to a second-order curve, increase.
R. D. Blake and J. R. Fresco (1966).	$n=2$ under the conditions where only poly (A+U) is formed.
J. G. Wetmur and N. Davidson (1968).	$n=2$ results reported just to 75% of the complete transformation.
R. J. Britten and D. E. Kohne (1968).	$n=2$.

D. RENATURATION MECHANISMS OF DNA AND SYNTHETIC POLYNUCLEOTIDES

The mechanism most frequently suggested for the renaturation of DNA as well as the synthetic polynucleotides is a two step transformation: nucleation followed by a zippering-up (Flory, 1961; Kallenbach *et al.*, 1963; Marmur and Doty, 1961; Ross and Sturtevant, 1960, 1962; Studier, 1969; Subirana and Doty, 1966; Wetmur and Davidson, 1968).

Nucleation is the formation of a minimum number of complementary base pairs permitting the strands to remain face to face. The fact that the total process seems to be second order suggests that this first step, a bimolecular process, is slow. The zippering-up, a unimolecular process, would in contrast be very rapid.

A certain number of complementary hypotheses have been made in order to explain deviations from second-order kinetics. Cavalieri and collaborators in studying DNA (Cavalieri *et al.*, 1962) imagined a mechanism involving two sorts of molecules: one being a single strand S and the other with strands partially separated (NS). In contrast, Thrower and Peacoke (1966) who also studied DNA, explained previous results using purely experimental considerations. Ross and Sturtevant (1960) put forward three possible hypotheses to make the deviation from second-order kinetics observed during the formation of poly (A+U) in solution of low ionic strength comprehensible:

The rate of cooperative ordering is much slower than that of nucleation.
The base pairs are randomly bound during nucleation.