PHYSICO-CHEMICAL PROPERTIES OF NUCLEIC ACIDS

Volume I

edited by Jules Duchesne

Physico-chemical Properties of Nucleic Acids

edited by J. DUCHESNE
Department of Molecular and Atomic Physics
University of Liège
Belgium

VOLUME 1: *Electrical, optical and magnetic properties of nucleic acids and components*

ACADEMIC PRESS
London · New York
1973

ACADEMIC PRESS INC. (LONDON) LTD.
24/28 Oval Road
London NW1

United States Edition published by
ACADEMIC PRESS INC.
111 Fifth Avenue
New York, New York 10003

Library of Congress Catalog Card Number: 72-84360
ISBN: 0-12-222901-0

PRINTED IN GREAT BRITAIN BY
Adlard and Son Ltd., Bartholomew Press, Dorking

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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

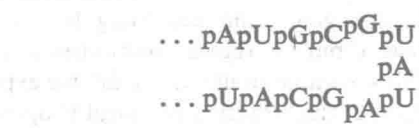
The chemist, if he encounters a new substance, is accustomed to look first for its macroscopic properties in order to correlate them with underlying structural features. Such properties instantaneously suggest to him some picture of the molecule, and often it is only the material quality which stimulated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

... pApUpGpCpGpUpApUpApGpCpApUp ...

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



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 realization 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 twenty 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-organization 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 organization 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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CHAPTER 1

The Dipole Moments of Purines and Pyrimidines

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Department of Organic Chemistry, Hebrew University, Jerusalem

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I. Introduction

The dipole moment of an organic (or inorganic) molecule is a vector (Smith, 1955; Boettcher, 1952; Smyth, 1955; Mirkin *et al.*, 1970) which, in a reasonable approximation, can be considered as the result of the individual moments of all bonds in the molecule combined, taking into account the angles the various bonds form with each other. Thus, if these angles are known, one is able to determine "bond moments", or—more important—if the bond moments are known, one can determine angles in the molecule. Therefore, dipole moments can be used, *inter alia*, for the assignment of configurations to geometrical isomers and of conformations—if one can assume that the latter are stable and do not change rapidly. Furthermore, it is obvious that the knowledge of the dipole moment of an organic molecule can be used to detect the contribution of zwitterionic resonance forms to the ground state of the molecule; it will permit some insight into the manner in which the molecule will associate with other molecules. As it happens, in the pyrimidine and purine series in which such association is physically and biologically important, it has been shown (Pullman, 1968) that the dipole moments play only a minor part in this phenomenon.

Because of the possibility that the dipole moment of a molecule causes the latter to form aggregates and to undergo solvation, especially in polar solvents, the dipole moments are measured either in the gas phase—which is mainly done for small molecules—or in solution in non-polar solvents. This is a

serious limitation specifically for such relatively complex molecules as pyrimidines, purines or their derivatives which occur in nature. It should be mentioned that recently (Myers and Sun, 1966) a method has been suggested to derive the dipole moments from measurements in mixtures of polar and non-polar solvents, but this method has not yet found wider application, though it opens many new possibilities.

Modern quantum-chemical methods have made it possible to calculate the dipole moments of even complex molecules (Pullman and Pullman, 1952). In these cases, the total dipole moment is separated into that stemming from the distribution of the π-electrons and that representing the polarisation of the σ-bonds, these two being calculated independently. In both calculations, the geometry of the molecule, *inter alia*, has to be known or at least guessed well; such guesses can be improved by iterative procedures. It has been found that the method of linear combination of atomic orbitals (LCAO) gives generally good agreement between theory and experiment and that more refined methods of calculation do not substantially improve that agreement.

II. Quantum-chemical Calculations

Calculations on the dipole moments of some of the naturally occurring pyrimidine and purine bases have been carried out by many methods, from the simple Hückel procedure and later the Pariser–Parr–Pople (PPP) method (Veillard and Pullman, 1963) to the more refined ones, such as the so-called Extended Hückel Theory and the Iterative Extended Hückel Theory and finally the CNDO/2 procedure (Pople and Segal, 1965), one of the "self-consistent field" methods, which involves all valence-electrons (Pullman, 1969). Table I summarises the results obtained by various methods for the moment μ and angle θ which the calculated moment forms with the vertical axis of the molecule (counted counterclockwise) (Berthod *et al.*, 1966; Lindner *et al.*, 1966; Rein *et al.*, 1966; de Voe and Tinoco, 1962; Pullman *et al.*, 1968a; Geissner-Prettre and Pullman, 1968; Mely and Pullman, 1968). Looking at the μ_{total} values, we can see that whilst their trend and the angle θ are very similar in all calculations, the variation in the absolute values of the dipole moments is very large. The trend and the angle are largely due to the π-moments which obviously do not vary much in the various methods. Experience has shown that the method which gives the results nearest to the measured values of the dipole moments, is the CNDO/2 method taking into account the non-sphericity of the atoms (Geissner-Prettre and Pullman, 1968). A recent study has shown that only a very slight improvement in the agreement between calculation and measurement can be achieved by deorthogonalising the orbitals in the CNDO/2 method ("CNDO/2D method") (Shillady *et al.*, 1971).

TABLE I

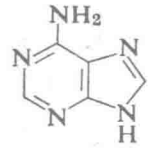
Calculated dipole moments of natural purine and pyrimidine bases

Compound	Hückel $\mu_\sigma+\mu_\pi$[a]	SCF $\pi+\sigma$	MO–LCAO–SCF PPP+Ohno extension[b] μ_π	μ_σ	μ_{tot}	Extended Hückel μ	θ	Iterative extended Hückel[c] $\pi+\sigma+$ lone pair	Iterative extended Hückel[d] μ_σ	θ	μ_π	θ	μ_{hyb}	θ	μ_{tot}	θ
Pyrimidine	—	—	0·80	0·72	1·52	—	—	—	—	—	—	—	—	—	—	—
Uracil	3·5	4·0	—	—	—	12·3	43	4·20	12·16	75·3	4·76	19·4	1·39	34·0	7·63	35·7
Cytosine	8·0	7·1	—	—	—	16·5	109	6·69	2·39	106·0	8·12	127·4	3·48	90·0	12·43	124·6
Thymine	—	4·0	—	—	—	—	—	4·53	—	—	—	—	—	—	—	—
Purine	—	—	—	—	—	—	—	—	0·53	172·4	5·11	4·76	2·48	34·8	7·22	56·6
Adenine	2·8	2·3	—	—	—	5·1	65	3·61	0·48	−1·2	3·94	132·3	1·76	50·4	4·39	104·3
Guanine	6·9	7·2	—	—	—	16·5	−14	8·73	0·66	13·0	11·2	−34·8	2·85	−32·4	145·0	32·4

Compound	CNDO/2[e] μ_σ	θ	μ_π	θ	$\mu_\sigma+\mu_\sigma$	θ	*ab initio* calculations[f] Exact value μ_σ	θ	μ_π	θ	μ_{tot}	θ	Point charge approx. μ_σ	μ_π	μ_{tot}	θ	PPP-calc. $\mu_\pi+\mu_\sigma$[g] μ_{tot}	θ
Pyrimidine	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Uracil	0·70	87	4·20	29	4·61	36	—	—	—	—	—	—	—	—	—	—	3·3	35
Cytosine	1·41	75	6·39	108	7·61	112	1·29	68	5·76	111	6·76	103	4·32	10·38	14·55	116	7·1	110
Thymine	0·64	86	3·94	33	4·35	39	—	—	—	—	4·22	50	—	—	10·13	57		
Purine	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Adenine	0·71	27	2·34	75	2·86	64	0·77	82	2·46	101	2·83	93	0·99	4·55	4·55	91	2·0	73
Guanine	0·28	63	7·27	−29	7·26	−27	—	—	—	—	—	—	—	—	—	—	7·2	−36

[a] de Voe and Tinoco (1962); [b] Lindner *et al.* (1966); [c] Rein *et al.* (1968); [d] Pullman *et al.* (1968a); [e] Geissner-Prettre and Pullman (1968); [f] Mely and Pullman (1969); [g] Pullman (1969).

In Table I it is assumed that the formula of adenine (I) is the classical one, viz. the "$N_{(9)}H$ amine" (see pp. 6–7).



I

However, it has been calculated that—rather unexpectedly—the various possible tautomeric forms of purine and its derivatives should have different dipole moments—so that, if one could isolate the various tautomers, their structure could be determined unequivocally by dipole moment measurement.

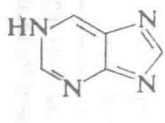
TABLE II

Calculated dipole moments for the purine (II) tautomers[a]

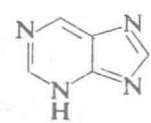
Tautomer	PPP–DBP method				CNDO method				
	μ_σ	μ_π	μ_{tot}	θ	μ_σ	μ_π	μ_{hyb}[b]	μ_{tot}	θ
$N_{(1)}H$ (IIa)	1·44	4·21	5·38	241°	2·11	5·98	3·02	6·75	241°
$N_{(3)}H$ (IIb)	0·29	3·24	3·38	320°	1·90	4·72	1·68	4·19	320°
$N_{(7)}H$ (IIc)	1·82	3·79	5·30	153°	1·20	3·94	3·38	6·08	150°
$N_{(9)}H$ (IId)	0·64	3·05	3·68	46°	1·43	3·60	2·02	4·19	45°

[a] θ is the angle with the C_4–C_5 axis, counted counterclockwise; [b] μ_{hyb} = hybridisation moment.

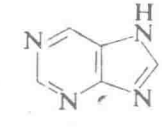
In the case of purine(II) itself, two methods have been used for the calculations: a PPP-treatment for the π-electrons, combined with a Del Re–Berthod–Pullman (DBP) procedure for the σ-electrons (Pullman and Pullman, 1968), and recently the CNDO/2 treatment referred to previously. Table II shows the results for the following four tautomers:



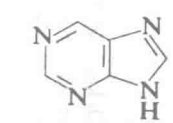
$N_{(1)}H$
IIa



$N_{(3)}H$
IIb



$N_{(7)}H$
IIc

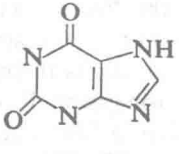


$N_{(9)}H$
IId

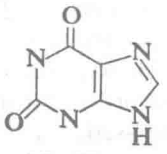
The figures for the $N_{(9)}H$ tautomer are based on the assumption that its geometry is that described by Spencer (1959); as already pointed out, other assumptions may cause slight changes of the figures.

It is striking that both calculations lead to the separation of the four tautomers into two groups: $N_{(1)}H$ and $N_{(7)}H$ have higher moments than $N_{(3)}H$ and $N_{(9)}H$ (Pullman *et al.*, 1968a, 1969a, 1970).

Analogous calculations have been made for xanthine (III), for which two tautomers are possible, $N_{(7)}H$ and $N_{(9)}H$ (Table III) (Pullman *et al.*, 1969a):



$N_{(7)}H$
IIIa



$N_{(9)}H$
IIIb

TABLE III

Calculated dipole moments for xanthine (III) tautomers (CNDO/2 method)

Tautomer	μ_σ	μ_π	μ_{hyb}	μ_{tot}	θ
$N_{(7)}H$ (IIIa)	0·95	4·13	1·38	3·97	99
$N_{(9)}H$ (IIIb)	2·18	−6·66	3·52	7·86	24

TABLE IV

Calculated dipole moments of monohydroxypurines (counted counterclockwise with regard to the C_4–C_5 axis)

Compound	SCF CI method		CNDO method	
	μ (D)	θ	μ (D)	θ
6-Hydroxypurine (hypoxanthine)				
$N_{(1)}H$–$N_{(9)}H$ tautomer (IVa)	5·6	−16	5·9	−16
$N_{(3)}N$–$N_{(9)}H$ tautomer (IVb)	10·5	11	11·8	11
$N_{(1)}H$–$N_{(7)}H$ tautomer (IVc)	2·6	−157	2·6	−156
$N_{(3)}N$–$H_{(7)}H$ tautomer (IVd)	3·6	41	4·9	36
8-Hydroxypurine				
$N_{(7)}H$–$N_{(1)}H$ tautomer (Va)	9·4	−128	11·2	−127
$N_{(7)}H$–$N_{(3)}H$ tautomer (Vb)	4·8	−93	5·7	−94
$N_{(7)}H$–$N_{(9)}H$ tautomer (Vc)	2·3	−118	1·4	−129
2-Hydroxypurine				
$N_{(1)}H$–$N_{(3)}H$ tautomer (VIa)	2·0	−163	2·3	−140
$N_{(1)}H$–$N_{(7)}H$ tautomer (VIb)	9·1	153	10·9	154
$N_{(1)}H$–$N_{(9)}H$ tautomer (VIc)	4·4	113	5·0	119
$N_{(3)}H$–$N_{(7)}H$ tautomer (VId)	8·4	122	9·1	119
$N_{(3)}H$–$N_{(9)}H$ tautomer (VIe)	8·0	62	8·8	61