THE PHYSICO-CHEMICAL BEHAVIOUR OF
POLYNUCLEOTIDES IN NON-AQUEOUS SOLUTION.

Josephine Anne Weigold, B.Sc. (Adelaide)

Department of Physical and Inorganic Chemistry,
The University of Adelaide,
South Australia.

Thesis presented for the degree of
Doctor of Philosophy.

April, 1965
# CONTENTS

<table>
<thead>
<tr>
<th>Chapter I</th>
<th>INTRODUCTION</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter II</th>
<th>ENZYMIC SYNTHESIS AND THE STRUCTURE OF POLYRIBONUCLEOTIDES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Polynucleotide Phosphorylase</td>
<td>10</td>
</tr>
<tr>
<td>(i) Reactions promoted</td>
<td>10</td>
</tr>
<tr>
<td>(ii) Distribution</td>
<td>11</td>
</tr>
<tr>
<td>(iii) Enzyme properties</td>
<td>12</td>
</tr>
<tr>
<td>(iv) Specificity</td>
<td>13</td>
</tr>
<tr>
<td>(v) Primer requirements</td>
<td>14</td>
</tr>
<tr>
<td>(vi) Isolation</td>
<td>18</td>
</tr>
<tr>
<td>a) from <em>Azotobacter agilis</em></td>
<td>18</td>
</tr>
<tr>
<td>b) from <em>Micrococcus lysodeikticus</em></td>
<td>19</td>
</tr>
<tr>
<td>2. Synthesis and isolation of polynucleotides</td>
<td>20</td>
</tr>
<tr>
<td>3. Polynucleotide structure</td>
<td>22</td>
</tr>
<tr>
<td>(i) Primary</td>
<td>22</td>
</tr>
<tr>
<td>(ii) Secondary</td>
<td>24</td>
</tr>
</tbody>
</table>
Chapter III

THE PHYSICO-CHEMICAL BEHAVIOUR OF

POLYNUCLEOTIDES IN AQUEOUS SOLUTION

WITH PARTICULAR REFERENCE TO POLY-A

1. Introduction 31

2. The size and shape of polynucleotides
   from light scattering data 33

3. Hydrodynamic properties 36
   (i) Viscosity 38
   (ii) Sedimentation 43
   (iii) Molecular weights from hydrodynamic data 45

4. Optical properties 47
   (i) Ultraviolet absorption spectra 47
       a) Molar absorbance 48
       b) Hypochromicity 51
       c) Effect of pH 53
       d) Effect of temperature 54
       e) Effect of ionic strength 57
   (ii) Optical rotation and optical rotatory dispersion 57
   (iii) Infrared spectra 61
   (iv) Nuclear magnetic resonance 62

5. Conclusions 64
Chapter IV

THE PHYSICO-CHEMICAL BEHAVIOUR OF

POLYNUCLEOTIDES IN NON-AQUEOUS SOLUTIONS,

WITH PARTICULAR REFERENCE TO POLY-A.

1. Introduction 67

2. The solubility of polynucleotides
   and nucleic acids in non-aqueous solvents 69
   (i) Alkali metal salts 69
   (ii) Quaternary ammonium salts 70

3. Macromolecular properties 75
   (i) Viscosity 76
      a) Time dependence 78
      b) Concentration dependence 81
      c) Dependence on concentration
         of added salt 83
   (ii) Sedimentation 84

4. Optical properties 90
   (i) Ultraviolet absorption spectra 90
   (ii) Optical rotation and optical
        rotatory dispersion 95

5. The effect of solution in non-aqueous
   solvents on the nature and properties of
   the recovered polynucleotides and nucleic
   acids in aqueous solutions 99

6. General discussion 106
Chapter V

EXPERIMENTAL

1. Bacteriological methods 111
2. Preparation of polynucleotide phosphorylase 112
   (i) from A. vinelandii 112
   (ii) from M. lysodeikticus 116
3. Enzyme assay 116
4. Protein estimation 117
   (i) Biuret method 117
   (ii) Spectrophotometric method 118
   (iii) Folin-Ciocalteu method 118
5. Polynucleotide synthesis 119
6. Isolation and purification of polynucleotides 120
7. Purification of solvents 122
8. Preparation of quaternary ammonium salts of polynucleotides and nucleic acids 123
9. Spectrophotometry 123
10. Optical rotatory dispersion 126
11. Sedimentation 126
12. Viscometry 127

BIBLIOGRAPHY
SUMMARY

The recognition of the biological importance of nucleic acids, both ribo- and deoxyribo-nucleic acids, has prompted the investigation of their physico-chemical properties \textit{in vitro}, in attempts to elucidate the configuration, the size and the mode of biological activity of these biopolymers. The discovery of several enzyme systems, capable of promoting polynucleotide synthesis, greatly assisted in these studies, since synthetic polynucleotides having limited compositions could then be studied. Examination of the helical structures formed by synthetic homopolyribonucleotides has provided considerable insight into the problem of the specificity and interactions of naturally-occurring nucleic acids.

Although the biological activity of nucleic acids is intimately related to the configuration and interactions of the polymer under the conditions existing \textit{in vivo}, physico-chemical investigations have largely been carried out using aqueous salt solutions which do not necessarily resemble the biological conditions. Even in these simplified systems it is difficult to obtain accurate quantitative data on
polynucleotides or nucleic acids. This results, not only from the variability in the configuration, as exemplified by helix-coil transitions or denaturation, and from the macromolecular nature and the frequent heterogeneity of these compounds, but is also due to their polyelectrolyte behaviour arising from the charged nature of the polymeric backbone. This behaviour severely hampers the characterization of differing polynucleotide or nucleic acid samples, and virtually precludes an unambiguous assessment of polymer shape and size or correlation of physico-chemical properties with biological function.

The possibility of using non-aqueous solutions for characterization of polymer samples has been further investigated in the present work, with particular reference to the hexadecyltrimethyl ammonium salt of polyadenylic acid (poly-A-Q) in ethanol solution. Although these systems are, in principle, more easily reproduced and defined, many of the problems encountered in aqueous systems are also evident under non-aqueous conditions. This is found to apply particularly to the uncertainty of the extent of the helix-coil transition and to polyelectrolyte behaviour. In general, the results
iii.

In general, the results obtained from sedimentation, viscosity and optical measurements for poly-A-Q and DNA-Q in ethanol are consistent with the polymer chains existing in a collapsed random coil configuration. However, the physico-chemical properties of polynucleotides and nucleic acids in non-aqueous solution were found to be sensitive to the presence of low concentrations of ionic impurities. Thus, it has been concluded that non-aqueous solutions of these biopolymers, either in the form of the simple inorganic salts or as the quaternary ammonium salts, are not suitable for use in the characterization of various samples.