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OFFICE OF NAVAL RESEARCH

FINAL REPORT

By

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Project NR 304-155

Contracts N7onr-28509 and Nonr-1202(19)

September 1, 1951 to June 30, 1963

Report Submitted July 5, 1963

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I. OBJECTIVES

The original project title was "The Properties and Mechanism of Formation of Gel Structures from Proteins and other Macromolecules". In the second Contract covering this project, it was modified to "Viscoelastic Properties of Biological Macromolecules". *

The original objectives were stated as follows:

- (1) A study of the mechanism of conversion of fibrinogen to fibrin, with emphasis on the properties of intermediates.
- (2) a delineation of the conditions under which nucleic acid exists as a gel, and a description of the properties of its gels.
- (3) a description of the properties of gels formed from denatured proteins. **

In 1955, the following was added:

- (4) A study of the mechanical properties of other gel-forming systems, especially solutions of gelatin and cellulose derivatives, in both gelatinous and non-gelatinous states. ***

Under the second contract, the following objectives were stated:

- (5) Information concerning the internal flexibility of protein molecules in both native and denatured states, from viscoelastic measurements in dilute solutions.
- (6) Similar investigations of synthetic polymers of simpler structure to aid in interpreting the results of measurements on proteins.
- (7) Similar investigations of other biological macromolecules. performed

Extensive results have been obtained for (1), (2), (4), and (6), and to a lesser extent for (5). The evolution of the research did not permit investigation of (3) and (7). **

II. SUMMARY OF RESULTS ACHIEVED

(1) Conversion of Fibrinogen to Fibrin

The size, shape, and thermodynamic interactions of bovine fibrinogen were investigated by light scattering and sedimentation and diffusion. The two stages of polymerization of activated fibrinogen -- to intermediate polymers and subsequently to fibrin -- were investigated under a variety of conditions: from pH 5.5 to 10.2; with hexamethylene glycol and various alkali halides as inhibitors, and with no inhibitor; in the presence of calcium; and at various ionic strengths. By sedimentation and light scattering measurements, the size, shape, thermodynamic interactions, dissociation, and reassociation of the

intermediate polymers were studied under various conditions. Their rodlike character was supported by measurements of non-Newtonian flow and transverse wave propagation. Measurements of electrical birefringence of solutions of fibrinogen and activated fibrinogen provided evidence about the change in charge pattern accompanying activation, and the effect of pH and added urea on the dipole moment and rotary diffusion coefficient. ↗

(2) Properties of Nucleic Acid

Solutions of sodium desoxyribonucleate (molecular weight about 8 million) were studied by the falling sphere viscosity method with miniature glass spheres and the wave propagation method to determine its mechanical properties at pH 6.0, both in 0.2 M sodium chloride and in salt-free solution. From the dependence of viscosity on shear rate, concentration, and temperature, as well as the magnitude of the dynamic rigidity and its dependence on frequency, concentration, and temperature, the relaxation distribution function was calculated and found to resemble those of flexible polymers, revealing cooperative configurational changes and coupling by entanglement in moderately concentrated solution; while in very dilute solution the mechanical behavior resembled more that predicted for stiff thin rods.

(3) Gels from Denatured Proteins

(Not investigated during term of project)

(4) Mechanical Properties of Other Gel-Forming Systems

Measurements of the viscoelastic properties of the system cellulose nitrate-diethyl phthalate both above and below the gel temperature were made by electromagnetic transducers at audiofrequencies, a torsion pendulum at low frequencies, and creep at long time intervals. Dynamic viscoelastic measurements were also made on several gels of polyvinyl chloride in solvents of widely different viscosities. In some of these, the mechanical properties were followed in detail through the course of gelation. From analysis of these data, conclusions were drawn concerning the local effective viscosity controlling motion of network strands, and the importance of gel strands with loose ends. Improved methods for approximate interconversion of data for various time-dependent mechanical properties were devised. The viscosities of concentrated solutions of certain polyelectrolytes were studied as a function of degree of neutralization, revealing certain strong interactions between hydrophobic groups in aqueous solutions resulting in pseudo-gelatinous mechanical behavior. The application of ladder network models for theoretical interpretation of viscoelastic properties was examined.

(5) Internal Flexibility of Protein Molecules from Viscoelastic Measurements

Preliminary experiments on the dynamic shear response of bovine serum albumin in aqueous glycerol solution have shown a definite elastic effect at lower frequencies, though the data are not yet sufficient to distinguish between effects of orientation and deformation and thus to gauge the internal flexibility.

(6) Internal Flexibility of Synthetic Polymer Molecules from Viscoelastic Measurements

Dynamic viscoelastic measurements were made on dilute polystyrene solutions in solvents of high viscosity, and provided the first experimental confirmation of the Zimm theory. Further measurements on different polystyrenes and polyisobutylenes revealed deviations from the Zimm theory which depended on molecular weight, concentration, and the thermodynamic properties of the solvent. An extended theory was developed which explains these effects. Similar measurements on dilute solutions of poly- γ -benzyl-L-glutamate in the helical form showed a behavior intermediate between the predictions of the Zimm theory for flexible coils and the Kirkwood-Auer theory for rigid rods, indicating rods with some degree of flexibility; in contrast, the behavior of this polymer in the random coil form was consistent with the theory of Zimm. Some exploratory measurements of the electrical birefringence of poly- γ -benzyl-L-glutamate in its helical form were also made.

(7) Internal Flexibility of Other Biological Macromolecules from Viscoelastic Measurements

(Not investigated during term of project)

These results, obtained over a period of nearly twelve years, cover a wide variety of properties of macromolecules of both natural and synthetic origin. They have provided information concerning the size, shape, flexibility, and viscoelastic response of individual molecules in dilute solution; their associations and entanglements to form extended structures; and the mechanical behavior of entangled and cross-linked systems, most of which can be loosely described as "gelatinous" but differ in detailed properties depending on the nature and disposition of the intermolecular linkages and the flexibility of the network strands.

III. PERSONNEL

The following persons, besides the Principal Investigator, have held appointments on the project:

Name	Degree	Present Position
<u>Graduate Students</u>		
Donald J. Plazek	Ph.D., 1956	Research Staff, Mellon Institute
Theodore P. Yin	Ph.D., 1960	Research Staff, E. I. du Pont de Nemours and Company
William H. Glaze	M.S., 1957	Dept. of Chemistry, North Texas State University
Thomas E. Newlin	M.S., 1959	Research Staff, Eli Lilly and Company
Meyer H. Birnboim	Ph.D., 1961	Research Staff, Mellon Institute
Robert A. Stratton	Ph.D., 1962	Research Staff, Mobil Chemical Company
Stephen D. Morton	Ph.D., 1962	Dept. of Chemistry, Otterbein College
J. E. Frederick		Ph.D. Candidate
John W. Allis		Ph.D. Candidate
<u>Postdoctorate Associates</u>		
Sidney Katz		Dept. of Biophysics, University of Buffalo
Edward F. Casassa		Research Staff, Mellon Institute
Walter Dannhauser		Dept. of Chemistry, University of Buffalo
Stuart E. Lovell		Theoretical Chemistry Institute, University of Wisconsin
Nicholas W. Tschoegl		Research Staff, Stanford Research Institute
<u>Foreign Associate</u>		
Kazuhiko Ninomiya		Research Staff, Japan Synthetic Rubber Company

The following graduate students participated in the project without contract support:

Ignacio Tinoco, Jr.	Ph.D., 1954	Dept. of Chemistry, University of California, Berkeley
Irwin H. Billick	Ph.D., 1955	National Bureau of Standards
Frances H. Webb	Ph.D., 1956	Dept. of Biochemistry, University of Wisconsin

IV. PUBLICATIONS

1. Sidney Shulman and Sidney Katz, "Dissolution of Fibrin by Neutral Inorganic Salts", Federation Proceedings, 11, 286 (1952).
2. Sidney Katz, Kurt Gutfreund, Sidney Shulman, and John D. Ferry, "The Conversion of Fibrinogen to Fibrin. X. Light Scattering Studies of Bovine Fibrinogen", J. Amer. Chem. Soc., 74, 5706-5709 (1952).
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5. Sidney Katz and John D. Ferry, "Viscosity and Rigidity of Solutions of Sodium Thymonucleate", J. Amer. Chem. Soc., 75, 1589-1593 (1953).
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18. Frances E. Helders and John D. Ferry, "Non-Newtonian Flow in Concentrated Solutions of Sodium Desoxyribonucleate," J. Phys. Chem., 60, 1536-1542 (1956).
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33. Nicholas W. Tschoegl and John D. Ferry, "Viskoelastische Eigenschaften von Polyisobutylenen in verdünnten Lösungen," Kolloid-Z., 189, 37-40 (1963).
34. Nicholas W. Tschoegl, "Influence of Hydrodynamic Interaction on the Viscoelastic Properties of Dilute Polymer Solutions," J. Chem. Phys., in press.
35. Nicholas W. Tschoegl, "Influence of Hydrodynamic Interaction on the Viscoelastic Behavior of Dilute Polymer Solutions in Good Solvents," J. Chem. Phys., to be submitted.
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37. Nicholas W. Tschoegl and John D. Ferry, "Dynamic Mechanical Properties of Poly- γ -Benzyl-L-Glutamate in a Helicogenic Solvent," J. Amer. Chem. Soc., to be submitted.

38. J. E. Frederick, Nicholas W. Tschoegl, and John D. Ferry, "Dynamic Mechanical Properties of Dilute Solutions of Polystyrenes of Different Molecular Weights," J. Phys. Chem., to be submitted.

There were no previous technical reports on this project -- only annual and semi-annual progress reports.