Conformational Flexibility of a Myelin Protein†

M. A. Moscarello,* J. Gagnon, D. D. Wood, J. Anthony, and R. Epand

ABSTRACT: A protein, isolated from normal human myelin, assumed a predominantly α-helical or β conformation depending on the dialysis procedure from a phenol–acetic acid–urea solvent to water. The large b_0^{212} values found on optical rotary dispersion analysis in the phenol–urea solvent indicated a high percentage of helix. Removal of both phenol and urea in the first dialysis step resulted in a water-soluble preparation containing an appreciable amount of helix. However, removal of the phenol only in the first step followed by the subsequent removal of urea resulted in a preparation showing mainly β conformation. The conformational transition form α-helical to β form was also induced by heat. The change of ellipticity, $[\theta]_{222}$, with temperature decreased steadily from 40 to 90°. When the ^{131}I-labeled protein was heated, the transition to the β form was completed after 5 min at 100° and was not reversed by cooling. By equilibrium ultracentrifugation the molecular weight of the α-helical form was 86,000, while that of the β form was greater than 500,000. In 98% formic acid or 0.5% sodium dodecyl sulfate the molecular weights were reduced to 28,000 and 24,000, respectively.

The major chemical constituents of membranes, lipids, and proteins vary in proportion depending on the nature of the membrane. The myelin membrane with which we are concerned contains approximately 70–75% lipid; the remainder is protein.

Until recent years most chemical studies on the structure of membranes were concerned with the isolation and characterization of lipids, since they were readily extracted and analyzed by a number of well-established techniques. The proteins have been more difficult to study because they have been generally difficult to solubilize in aqueous media. Nevertheless, some progress has been made and membrane proteins are being studied in a number of laboratories.

The myelin membrane of the nerve has several advantages over other membranes. Large amounts of myelin can be readily obtained by a variety of methods (Laatsch *et al.*, 1962; Autilio *et al.*, 1964; Lowden *et al.*, 1967). One of the myelin proteins, the encephalitogenic protein, which has potent biological activity, has been extensively characterized and a sequence has been published (Eylar *et al.*, 1971; Westall *et al.*, 1971; Kibler *et al.*, 1969; Carnegie, 1971). The encephalitogenic protein is readily extracted and is soluble in aqueous media; however, other myelin proteins, including the one described here, are more intractable.

One such intractable protein fraction has been the proteolipid fraction (Folch and Lees, 1951) which consisted of a mixture of proteins and lipids soluble in organic solvents. In further studies the majority of the lipid was removed and the resulting protein components were dialyzed into water from chloroform–methanol (Stoffyn and Folch-Pi, 1971).

We have used a different method to obtain a protein from myelin with properties similar to that of proteolipid (Gagnon *et al.*, 1971; Wood *et al.*, 1971). It was found to undergo an interesting conformational transition from α-helical to β conformation (Anthony and Moscarello, 1971). The protein was insoluble in water, but could be dissolved in phenol–acetic acid containing 2 M urea and then transferred to water.

If phenol and urea were removed in a single step an α-helical circular dichroic (CD) spectrum was obtained. However, if phenol was removed first, followed by urea, a β-type spectrum was recorded. The assignment of α-helical and β to the spectra is qualitative since there are no appropriate models for the secondary structure of membrane proteins. A number of studies are under way to understand the significance of this conformational flexibility and the role such flexibility may play in the functioning of the myelin membrane.

Materials and Methods

Normal human myelin was prepared from white matter by the method of Lowden *et al.* (1967).

Lyophilized myelin was extracted first with 0.02 M mercaptoethanol followed by 0.2 N H_2SO_4. The residue was dissolved in acidified chloroform–methanol (1:1) containing 5% of 0.1 N HCl and fractionated on an LH-20 column. The protein eluted from the column was precipitated and lyophilized (Gagnon *et al.*, 1971).

Preparation of the Water-Soluble Form. The lyophilized protein (10 mg) was dissolved in 2 ml of phenol–acetic acid–water (3:1:1) containing 2 M urea. When dialyzed against 25% acetic acid followed by decreasing concentrations of acid, the α-helical form was obtained. If the dissolved protein was dialyzed against 50% acetic acid containing 2 M urea followed by 50, 25, 10, 5, and 0% acetic acid, the β conformation was formed. The dialysis procedure took 3 days.

Optical Rotary Dispersion (ORD) and Circular Dichroism (CD). ORD was done on a Jasco ORD/CD (Durrum Instruments, Palo Alto, Calif.). The Moffitt–Yang b_0^{212} values were calculated by the Moffitt equation (Moffitt–Yang, 1956) from plots of data obtained between 420 and 300 nm. Cells of 1-cm path length were used with protein concentrations varying between 0.75 and 1.3 mg/ml. Each value in Table I represents the mean of five determinations. For studies on the effect of the various stages of dialysis, samples were withdrawn at the appropriate stage from a single protein solution.

† From the Division of Biochemistry, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada, and the Department of Chemistry, University of Guelph, Guelph, Ontario, Canada. *Received March 5, 1973.* Financial support from the Multiple Sclerosis Society of Canada is gratefully acknowledged.

* Address correspondence to this author at the Hospital for Sick Children.

TABLE I: ORD Parameter b_0^{212} Calculated from Spectra Run between 420 and 300 nm.

Dialysis Stage	b_0^{212}	
	α-Helical Form	β Conformation
Phenol–acetic acid–water + 2 M urea	−440	−440
2 M urea in 50% acetic acid		−110
50% acetic acid		−200
25% acetic acid	−330	−200
10% acetic acid	−320	−160
5% acetic acid	−320	−180
Water	−320	−185

CD was done on a Cary 61 spectropolarimeter equipped with a heating block. Cells of 1.0-mm path length were used. Each sample was scanned twice. The spectra at higher temperatures were corrected for the dilution effect caused by thermal expansion of the solution.

Infrared Studies. A drop from a solution containing about 1 mg/ml of protein was placed on a 3 × 4 cm AgCl crystal 1 mm thick and stroked back and forth in one direction until complete evaporation had occurred. With a slowly evaporating solvent like water this operation was done under a lamp. A region where the film appeared homogeneous was chosen and a piece of black plastic with a square hole was placed over it. The crystal and plastic covers were mounted on the holder and scanned in a Beckman IR-20A.

Protein Measurements. Polyacrylamide gels (7.5%) were run by the method Takayama *et al.*, (1964). The water-soluble form of the protein was layered on top of the gels in 25% sucrose. Electrophoresis was carried out for 2 hr at 2 mA/tube.

Iodination. Iodination of the protein with ^{131}I was performed essentially by the method of Greenwood *et al.* (1963), except that the Sephadex column recommended for the removal of free iodine was not used. For iodination 1 mg of lyophilized protein was treated with 1.0 mCi of ^{131}I (4 μg of iodine per 100 mCi of ^{131}I). Since our protein was not soluble at the termination of the reaction, it was dialyzed extensively against distilled water until no ^{131}I was detected in the dialysate. The sac contents were lyophilized. The protein was dissolved in phenol–acetic acid–urea and dialyzed as described above so that a water-soluble preparation was obtained.

Ultracentrifugation Studies. Analytical ultracentrifugation was done in a Beckman Model E analytical ultracentrifuge at 20°. For equilibrium runs the ANJ rotor was used at 9000 rpm for 48 hr at 20° for the α and β forms of the protein. For the protein dissolved in 98% formic acid or 0.5% sodium dodecyl sulfate in 0.01 M phosphate buffer (pH 7.2), a speed of 44,000 rpm was used for 48 hr. For all runs a double sector Kel F centerpiece was used with an ultraviolet counterbalance. We are indebted to Dr. D. Kells, Department of Biochemistry, University of Toronto, for these runs.

Results

The α helical and β forms were prepared as described under Materials and Methods. The CD spectra of the two forms of the protein in water at pH 6.0 are shown in Figure 1. The α-helical form is shown in Figure 1A. Two troughs at 209 and 220 nm, typical of α-helical proteins and polyamino acids,

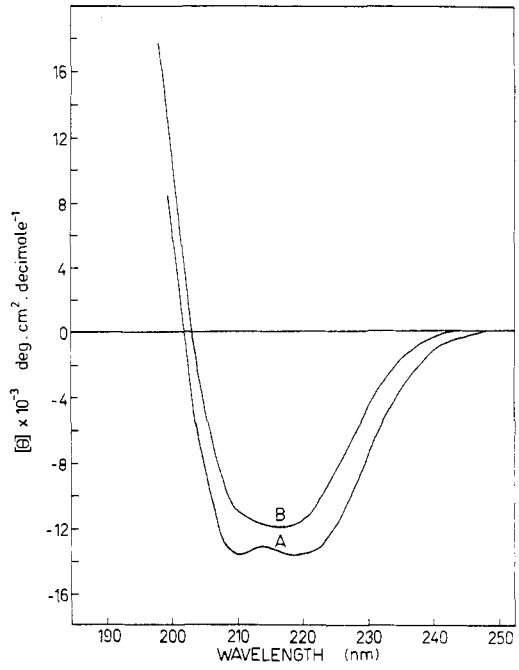

FIGURE 1: CD spectra for α-helical (A) and β forms (B) of the myelin protein in water (pH 6.0).

were recorded. The β form (Figure 1B) showed a single minimum at about 217 nm. In view of the uncertainty regarding the existence of suitable models for membrane proteins, a quantitative interpretation of the spectra was not made.

The infrared spectra of the two conformations are shown in Figure 2. The α form (Figure 2A) showed an infrared peak at 1650 cm^{-1} and a lesser peak at 1630 cm^{-1} representative of the α-helical and β forms, respectively. The infrared spectrum of the β form (Figure 2B) showed a major peak at 1630 cm^{-1} and a much smaller peak at 1650 cm^{-1}. The shoulder at 1690

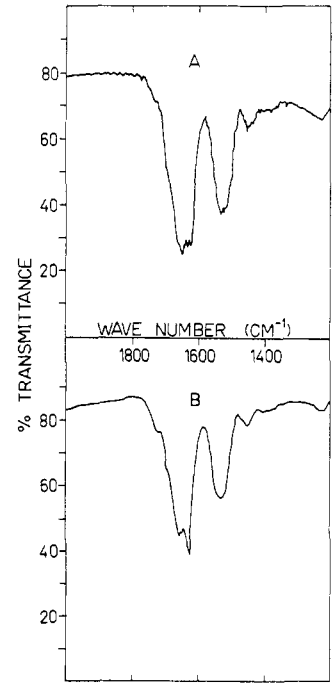

FIGURE 2: Infrared spectra of α-helical (A) and β forms (B) of the myelin protein. Films on AgCl crystals were prepared from water solution.

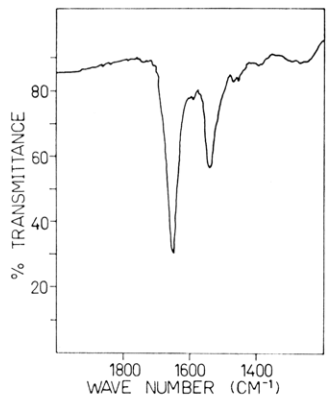

FIGURE 3: Infrared spectrum of the myelin protein prepared from a solution of the protein in phenol–acetic acid (2:1).

cm^{-1} was also attributed to the antiparallel β form (Miyazawa, 1960; Miyazawa and Blout, 1961).

The change in helical content during the various stages of dialysis was studied by ORD in the 420–300-nm range in an effort to determine if the protein adopted a random conformation at some stage. Samples were withdrawn at various times after the start of dialysis for ORD analysis. The b_0^{212} values were obtained from the Moffitt equation (Moffitt and Yang, 1956). At each stage in dialysis, determinations were made on five independent samples. These results are shown in Table I.

The b_0^{212} values in the starting solvent were high (−440) indicating a large proportion of helix, a b_0^{212} value of −630 representing 100% α helix (Yang, 1967). It decreased markedly to −100 in 50% acetic acid containing 2 M urea in the first stage of the dialysis procedure leading to the β form. In the next stage somewhat higher values were obtained (−200) and changed only slightly thereafter. When the procedure used to obtain the α-helical form was studied, the first stage involved a change from phenol–acetic acid–urea solvent to 25% acetic acid with b_0^{212} changing from −440 to −330 and was unaffected by the remainder of the dialysis procedure.

The infrared spectrum of the protein in phenol–acetic acid (2:1) is shown in Figure 3. A sharp peak at 1650 cm^{-1} was observed confirming the ORD data above from which the b_0^{212} values indicated a high percentage of α helix.

Equilibrium ultracentrifugation was carried out on the protein in water, in 98% formic acid, and in 0.5% sodium dodecyl sulfate in 0.01 M phosphate buffer (pH 7.2). The data are shown in Table II. A mol wt of 86,000 was obtained for the α-helical form in water and a mol wt of ~500,000 was obtained for the β form. In 98% formic acid or 0.5% sodium dodecyl sulfate in phosphate buffer (pH 7.2) values of 28,000 and 24,000 were obtained, respectively. The 28,000 value may represent the subunit; three subunits make up the α form, while the β form is an aggregate of many subunits.

TABLE II: Equilibrium Ultracentrifugation of the Protein in Water, Formic Acid, and Sodium Dodecyl Sulfate.

Sample	Mol Wt
α form in water	86,000
β form in water	~500,000
Protein in 98% formic acid	28,000
Protein in 0.01 M sodium phosphate buffer (pH 7.2) containing 0.5% sodium dodecyl sulfate	24,000

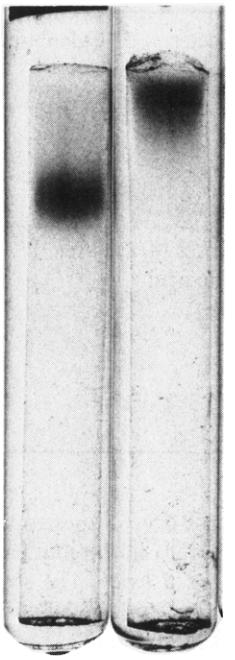

FIGURE 4: Polyacrylamide gel electrophoresis of α-helical (A) and β forms (B) of the myelin protein.

The difference in size of the α and β forms is reflected in the migration on 7.5% polyacrylamide gels (Takayama *et al.*, 1964). Typical gels are shown in Figure 4. The α form migrated well into the gel, while the β form moved just in below the surface.

The α-helical form was readily converted to the β form by heating. The protein at a concentration of 0.32 mg/ml in water was subjected to CD analysis in a Cary 61 spectropolarimeter equipped with a heating block. The spectra obtained are shown in Figure 5. Figure 5A represents the spectrum of the protein at 25°, which is largely α helical with minima at about 220 and 210 nm. When heated to 90° the spectrum shown in Figure 5C was obtained which is a β-type spectrum with a single minimum at about 217 nm. A partial recovery was observed when the solution was cooled to 25°, as shown in Figure 5B. The CD spectra provide evidence that the β structure was formed as a result of heating and not the result of heating followed by cooling.

The change in $[\theta]_{222}$ with temperature is shown in Figure 6. The spectropolarimeter was set at 222 nm and the rotation was recorded continously as a function of temperature. It can be seen that the spectrum remained constant up to about 40° after which a decrease in $[\theta]_{222}$ was observed with increasing temperature. The broadness of the transitions in the temperature range from 40 to 90° suggested that it was either not highly cooperative or that the enthalpy of the transition was small.

In order to obtain some quantitive measure of the conversion of the α-helical to the β form, the protein was iodinated with ^{131}I as described under Materials and Methods. The labeled protein was mixed with 9 mg of nonradioactive protein and the mixture was dialyzed *via* the α procedure. For the heating experiments 50 μl of the final solution was heated to the desired temperature, cooled, and applied to the top of a

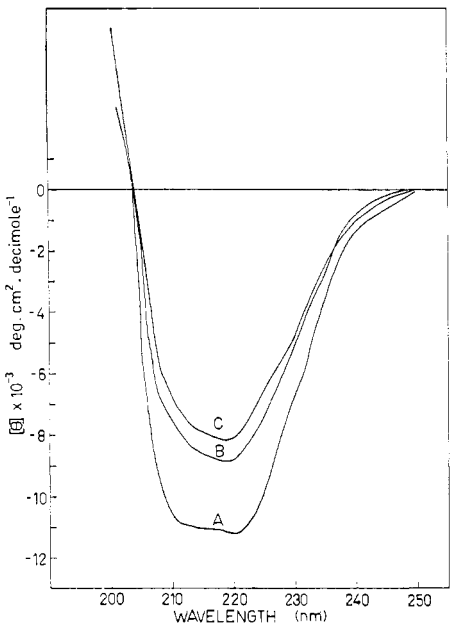

FIGURE 5: The effect of heating on the CD spectra of the α-helical form of the myelin protein: curve A, 25°; curve B, heated to 90° and then cooled to 25°; curve C, spectrum at 90°.

7.5% polyacrylamide gel. After running for 2 hr the gels were stained with Amido Black and destained with 7% acetic acid. The bands corresponding to the α-helical and β forms were sliced from the gels and counted in a Picker Autowell counter Model II. The results are shown in Figure 7. Complete conversion to the β form was achieved by 5 min at 90°, but somewhat longer times were required at the lower temperatures.

At zero time a ratio of about 3.4 was obtained indicating that a small amount of β was present on the gels. This was attributed to the effects of the iodination procedure which rendered the protein less soluble.

Discussion

The myelin protein isolated by us has considerable conformational flexibility. When isolated in acidified chloroform–methanol (1:1) the conformation was highly α helical (Anthony and Moscarello, 1971). However, the aqueous form showed much less helical content. In fact, after solubilization of the lyophilized protein in phenol–acetic acid–water (3:1:1) containing 2 M urea, the amount of helix was found to depend on the method used in dialysis. If the solution was dialyzed against 25% acid, removing both phenol and urea in one stage, the resulting water-soluble protein had an α-helical spectrum. It was clear from the CD spectra and infrared data that other conformations, in particular the β form, also contributed to the CD spectra of the α form. If, on the other hand, the protein dissolved in phenol–acetic acid–urea was dialyzed against 50% acetic acid containing 2 M urea in the first step a predominantly β spectrum was observed. It was possible to show these changes were reversible, *i.e.*, the β form was readily converted to the α-helical form by use of the appropriate dialysis procedure.

When the b_0^{212} values were calculated from ORD studies carried out during the various stages of dialysis, the procedure which yielded the β structure involved the passage of the protein through a stage with apparently less ordered structure (50% acetic acid + 2 M urea). Moffitt parameters of the order of magnitude reported here are in the range reported

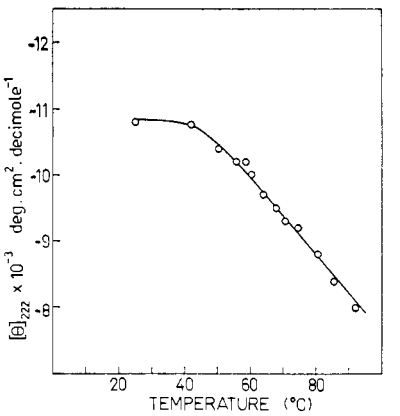

FIGURE 6: The change in $[\theta]_{222}$ as a function of temperature, recorded continuously from 25 to 90°.

(Yang, 1967) for several peptides and proteins containing the β structure. During the preparation of the α-helical conformation, the final form was attained during the first stage of the dialysis procedure, *i.e.*, 25% acetic acid. The gradual reduction in acid strength thereafter was required to maintain a soluble preparation.

The transition from α-helical to β form was readily induced by heat, showing a marked temperature dependence. When heated to 70–90°, a CD spectrum corresponding to a β structure was observed, the minima shifting from 220–222 to 217–218 nm. Cooling to room temperature brought only a small recovery. The minimum at 222 nm showed a continuous decrease with increasing temperature above 40°, showing that the change from α-helical to β form occurred continuously over the temperature range and did not show sharp transitions at particular temperatures.

The significance of this flexibility of conformation in proteins is not understood. It has been described in other protein systems. Scanu *et al.* (1969) and Dearborn and Wetlaufer (1969) have shown a temperature-dependent conformational change for human serum low-density lipoprotein. At low temperatures low-density lipoprotein has features of an α helix

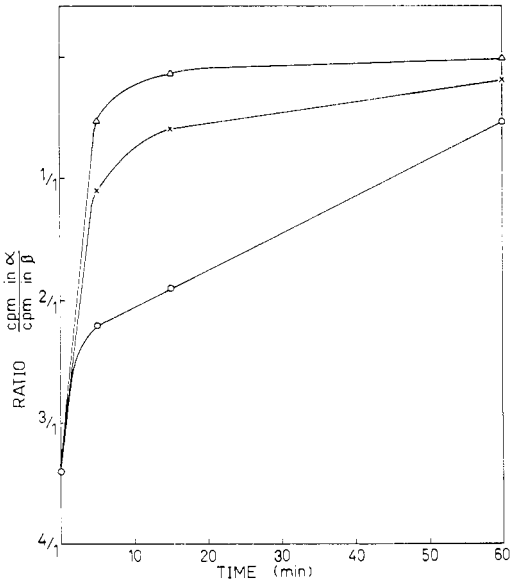

FIGURE 7: The conversion of α to β forms of ^{131}I-labeled myelin protein as a result of heating to 70, 80, or 90° for various times and separating on polyacrylamide gels.

and random coil with the appearance of a significant amount of β structure as the temperature was raised. The conformation was also found to be dependent on the method of preparation. When low-density lipoprotein was prepared by the method of Gotto *et al.* (1968), the presence of significant amounts of antiparallel β chain were found, with little or no α helix. Thus, the removal of the majority of the lipid from the lipoproteins resulted in a product showing significant conformational flexibility. It was postulated that the lipid may act to stabilize the structure.

The protein isolated in our laboratory has been freed of the vast majority of its associated lipid. The material is free of lipid phosphorus, sphingosine, glycerol, and galactose, but contains about 2% fatty acids which appear to be covalently bound (Gagnon *et al.*, 1971). It has a high content of apolar amino acids. This latter property with the associated fatty acids makes it a good candidate as bimodal protein.

This concept of bimodality of proteins was introduced by Colacicco (1969) and Vanderkooi and Green (1971) and has important implications for membrane structure. A bimodal protein is one with regions of apolar separated by short regions of polar amino acids. The apolar regions can fold up into helical conformations, thereby fitting into the hydrophobic environment of the membrane.

The myelin protein described here has two properties by which it can be considered a bimodal protein: (i) a high content of apolar residues already mentioned above and (ii) a flexibility of the polypeptide chain important in the folding of the protein, as expressed in the α-helix to β-conformational transition described in this article. It is too early to ascribe a functional significance to this transition in relation to the structure of the myelin membrane. It is tempting to speculate that such conformational flexibility may be part of the process of myelin degeneration.

Acknowledgments

We wish to thank Dr. R. Zand of the Biophysics Research Division of the University of Michigan, Ann Arbor, for assistance with the infrared measurements.

References

Anthony, J. A., and Moscarello, M. A. (1971), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 15*, 335.

Autilio, L., Norton, W. T., and Terry, R. (1964), *J. Neurochem. 11*, 17.

Carnegie, P. R. (1971), *Nature (London) 229*, 25.

Colacicco, G. (1969), *J. Colloid Interface Sci. 29*, 345.

Dearborn, D. G., and Wetlaufer, D. B. (1969), *Proc. Nat. Acad. Sci. U. S. 62*, 179.

Eylar, E. H., Brostoff, S., Hashim, G., Caccam, J., and Burnett, P. (1971), *J. Biol. Chem. 246*, 5770.

Folch, J., and Lees, M. (1951), *J. Biol. Chem. 191*, 807.

Gagnon, J., Finch, P. R., Wood, D. D., and Moscarello, M. A. (1971), *Biochemistry 10*, 4756.

Gotto, A. M., Levy, R. I., Rosenthal, A. S., Birnbaumer, M. E., and Fredrickson, D. S. (1968), *Biochem. Biophys. Res. Commun. 31*, 699.

Greenwood, F. C., Hunter, W. M., and Glover, J. S. (1963), *Biochem. J. 89*, 114.

Kibler, R. F., Shapira, R., McKneally, S., Jenkins, J., Selden, P., and Chou, F. (1969), *Science 164*, 577.

Laatsch, R. H., Kies, M. W., Gordon, S., and Alvord, E. C. (1962), *J. Exp. Med. 115*, 777.

Lowden, J. A., Moscarello, M. A., and Morecki, R. (1967), *Can. J. Biochem. 44*, 567.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.

Miyazawa, T. (1960), *J. Chem. Phys. 32*, 1647.

Miyazawa, T., and Blout, E. R. (1961), *J. Amer. Chem. Soc. 83*, 712.

Moffitt, W., and Yang, J. T. (1956), *Proc. Nat. Acad. Sci. U. S. 42*, 596.

Scanu, A., Pollard, H., Hirz, R., and Kothary, K. (1969), *Proc. Nat. Acad. Sci. U. S. 62*, 171.

Sherman, G., and Folch-Pi, J. (1970), *J. Neurochem. 17*, 597.

Stoffyn, P., and Folch-Pi, J. (1971), *Biochem. Biophys. Res. Commun. 44*, 157.

Takayama, K., MacLennan, D. H., Tzagoloff, A., and Stoner, D. C. (1964), *Arch. Biochem. Biophys. 114*, 223.

Vanderkooi, G., and Green, D. E. (1971), *BioScience 21*, 409.

Westall, F. C., Robinson, A. B., Caccam, J., Jackson, J., and Eylar, E. H. (1971), *Nature (London) 229*, 22.

Wood, D. D., Gagnon, J., Finch, P. R., and Moscarello, M. A. (1971), *Amer. Soc. Neurochem., Trans. 2, 1*, 117.

Yang, J. T., (1967), *in* Polyamino Acids, Fasman, G. D., Ed., New York, N. Y., Marcel Dekker, p 239.