

Circular Dichroism of Polypeptide Monolayers

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Received September 11, 1978; accepted October 12, 1978

The circular dichroism (CD) spectra of Langmuir-Blodgett monolayers and collapsed films of poly- γ -methyl-L-glutamate (PMG) and poly-L-alanine (PA) collected from the air-water interface of a Langmuir film balance have been determined. The polypeptide films were formed by spreading from a variety of solvents, followed by transfer to quartz plates for CD spectrophotometry. When all plates were aligned identically in the light beam of the spectropolarimeter, films of PMG and PA exhibited linear dichroism which could be used to determine the orientation of the polymer chains on the surface of the film balance. True CD spectra, free of linear component, were obtained on films oriented at numerous angles around the axis of the incident light beam. The CD spectra of films of PA and PMG in the α -helical conformation were consistent with the optical properties expected of polypeptides which have the helix axis perpendicular to the direction of propagation of light. PMG films gave spectra characteristic of the α -helix or β -conformation, depending on the spreading solvent used to form the monolayers. The pressure-area and surface potential curves, as well as the interpretation of the CD spectra of PMG and PA films, were all consistent with the results of previous investigations employing infrared spectroscopy. Details of preparing and mounting polypeptide monolayers for CD spectroscopy are given.

INTRODUCTION

Films of proteins spread at the air-water interface have been studied by the standard methods of surface chemistry for many years (1, 2), but investigations have been complicated by the fact that such molecules can exist in a variety of conformations (3). The earlier investigators were hampered by a lack of experimental methods for the determination of protein structure at the air-water interface.

Polypeptides have been employed recently as models for biological surfaces by a number of investigators (4-8). Monolayers may be prepared in which the polypeptide is in the α -helical or β -sheet conformation, depending on the spreading solvent employed (4-8). Poly-L-alanine (PA) and poly- γ -methyl-L-glutamate (PMG) spread from chloroform-dichloroacetic acid (DCA) gave

monolayers with the polypeptide in the α -helical conformation (4-8). Monolayers with the polypeptide largely in the β -conformation were formed when PMG was spread from pyridine-DCA (4) or pyridine-chloroform solvent which was rich in pyridine (8). Evidence for the presence of some α -helical structure in the film was obtained even when a spreading solvent containing pure pyridine was employed (8). Conformation studies on these systems have involved the use of infrared spectroscopy, deuterium exchange, and electron diffraction on collapsed films (5-7), and multiple internal reflection infrared spectroscopy of uncollapsed monolayers (4, 8).

Optical rotatory dispersion (ORD) and circular dichroism (CD) have been used for several years to study the conformation of polypeptides and proteins in solution (3, 9). Circular dichroism spectra of cast films of polypeptides (10-12) and monolayers of protein adsorbed from solution onto quartz

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plates (13) have also been determined. The possibility of determining CD spectra of monolayers deposited by the classical Langmuir-Blodgett technique (14) is readily apparent, especially with CD instruments capable of data manipulation and accumulation of multiple spectra.

The literature on the conformation of polypeptides at the air-water interface (4-8) provided examples for testing the applicability of circular dichroism to the study of monolayers. The results are reported below.

MATERIALS AND METHODS

Materials. The film balance was constructed of Lucite with trough dimensions of $50 \times 10 \times 1$ cm. Water circulating through a compartment beneath the trough provided temperature control, which was maintained at $21 \pm 0.5^\circ\text{C}$. Surface pressures were determined by the Wilhelmy plate technique with microscope coverslips and a Cahn RG electrobalance² (Cahn Instrument Co., Paramount, Calif.). The sensitivity of the surface balance was limited by vibrational noise to 10-20 mdyn. Surface potential measurements were made with an Am-241 ionizing electrode (Nuclear Radiation Developments Inc., Grand Island, N. Y.) and a Keithley 610C electrometer (Keithley Instruments, Inc., Cleveland, Ohio). The reference electrode was Ag/AgCl. Readings were reproducible to ± 10 mV. Drummond Dialomatic microdispensers (Drummond Scientific Co., Broomall, Penn.) were used for monolayer spreading. Quartz plates used for monolayer deposition were far UV silica (Optical Cell Co., Sykesville, Md.) with dimensions $12.5 \times 20 \times 1.25$ mm (W \times L \times T), hereafter referred to as the X, Y, and Z axes, respectively. The quartz plates with monolayers were positioned in the light beam of the CD instrument by either of two plate holders. Holder 1 aligned the faces of

the plates normal to the incident light beam with the Y axes all pointing in the same direction. In holder 2, the Y axes pointed in eight equally spaced intervals, 0, 45, 90, . . . 315° around the optical axis. The quartz plates were held in place by grooved brass blocks and springs mounted on the cell holders and designed so that only the outer 1-mm edge of each plate came in contact with metal. A hole through the blocks provided a path for the light beam. Masks with 7- to 9-mm openings attached to the brass end plates defined the light beam entering the holder from the monochromator. Circular dichroism spectra were recorded with a Jasco Model J-41C recording CD spectropolarimeter (Japan Spectroscopic Co. Ltd., Tokyo, Japan). The CD spectrum of poly- γ -methyl-L-glutamate in hexafluoroisopropanol agreed with published data (15, 16), showing the instrument to be in good calibration. Ultraviolet spectra were recorded with a nitrogen purged Cary-14 spectrophotometer with a 0.1 absorbance unit slidewire and adapted to record spectra to 180 nm.

All chemicals were reagent grade and used without further purification unless otherwise specified. Chloroform was treated with activated charcoal and acid alumina prior to use. Dichloroacetic acid was Fisher purified grade and vacuum distilled before use. Polypeptides from Sigma Chemical Co., St. Louis, Mo., were poly- γ -methyl-L-glutamate, MW = 147,000, and poly-L-alanine, MW "high."

Methods. Care and use of the film balance has been discussed in detail by Gaines (17). The Wilhelmy plates were cleaned with acid dichromate followed by thorough rinsing with distilled water and methanol and given a final cleaning and "hydrophilizing" in a RF oxygen Plasma for a few minutes immediately prior to use. The quartz plates used for monolayer depositions were treated similarly. Double-distilled water was used as the subphase. Spreading solvents were chloroform-DCA (99:1 vol) or chloro-

² Reference to brand or firm name does not constitute endorsement by the U. S. Department of Agriculture over others of a similar nature not mentioned.

form-pyridine-DCA (various mixtures) for PMG and chloroform-DCA (99:1) for PA, with polypeptide concentrations of 2 mg/ml or less. Monolayer spreading was by the direct application of numerous small (2–5 μ l) drops of the spreading solutions with a Drummond microdispenser.

The quartz plates were wrapped at one end with Teflon tape, leaving about 2.5 cm² area per plate exposed for monolayer deposition. The plates were mounted for dipping through the interface of the film balance with their Y axes perpendicular to the surface of the water. Before films were spread, the plates were lowered into the freshly cleaned subphase until the water touched the Teflon tape. The Wilhelmy plate was installed and the reference surface tension was determined. No buoyancy or end corrections were applied. The monolayer was then spread and allowed to equilibrate 15–20 min before compression. Typically, 20–30 μ g of polypeptide was spread over a surface area of 460 cm² and compressed manually at rates varying from 2 to 4 cm/min at low surface pressures to 0.5 cm/min for pressures above about 1 mN/m. At low surface pressure (high area), readings were taken immediately after compression. At high surface pressures, where a slight decrease in the pressure with time was noted, readings were arbitrarily taken 30 sec after each compression cycle. Upon reaching the desired transfer pressure, the film was held at constant area for 10 min after which the pressure was restored and transfer was started immediately. All plates "faced" the same direction during transfer and UV spectra and CD spectra runs. No plate was permitted to rotate about its Y axis with respect to the others. Collapsed films were obtained by removing the Wilhelmy plate, continuing compression until the two barriers were about 1.2 cm apart and sweeping a previously inserted quartz plate across the trough. Collapsed films were collected either upon completion of a Langmuir-Blodgett deposition or as separate runs.

The films were air or vacuum dried and the UV spectrum of the deposited monolayers was recorded between 400 and 180 nm.

The apparent CD spectra of collapsed or uncollapsed monolayers were obtained by first inserting the plate(s) into holder 1 and plotting the signal as a function of the angle of rotation of the film(s) about the axis of the incident light beam. These spectra contained contributions from both the circularly and linearly polarized light produced by the CD instrument. To obtain the true CD spectrum of uncollapsed monolayers, containing contributions from only the circularly polarized light, the plates were placed in holder 2 in orthogonal pairs and the CD signal accumulated for one-half the desired number of scans. The holder was then rotated 90° about the axis of the light beam and the remaining scans accumulated and the spectrum was plotted at the desired amplification. Spectra with more than eight orientations of the individual plates required multiple positioning of holder 2. For each position, data accumulation was interrupted at the mid-point and the holder was rotated 90°. For 16 orientations, for example, the holder was initially placed in the light beam with plates located at 0, 45, 90, . . . 315°. For reference, the position of the plate nearest the detector is underlined. The spectrum was then accumulated for one-fourth of the total number of scans desired. The sample was then rotated 90° and the second quarter of the data accumulated with plates located at 0, 45, 90, . . . 315°. The remainder of the data was accumulated in two equal periods with the plates located at new orientations; 22½, 67½, 112½, . . . 337½°, and 22½, 67½, 112½, . . . 337½°. The base line was accumulated and plotted in an identical manner with clean quartz plates. The CD spectra of collapsed monolayers, usually contained on single plates, were obtained by accumulating spectra at successive orientations of the plate around its incident light beam. For each orientation there was an orthogonal

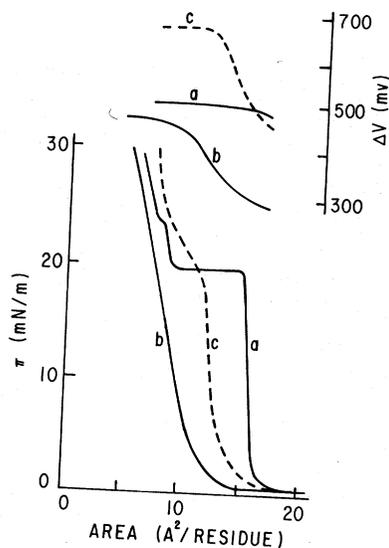


FIG. 1. Pressure (π)-area and surface potential (ΔV) isotherms of polypeptide monolayers. Polymethylglutamate spread from (a) chloroform-dichloroacetic acid (DCA) (99:1 vol); (b) pyridine-chloroform (98:2); (c) polyalanine spread from chloroform-DCA (99:1). Data points omitted for clarity.

partner, e.g., 0, 90, 180, 270; 45, 135°, etc. Base lines were obtained in an identical manner with clean plates and either applied as corrections to the accumulated spectra or plotted separately. The CD spectra of films are presented as ΔA , differential circular dichroic absorbance, or $\Delta\epsilon$, differential circular dichroic molar (residue) absorptivity, vs wavelength. For films where the transfer ratio (decrease in film area/plate area) suggested quantitative deposition of the monolayer onto the quartz plates, the results are presented in terms of $\Delta\epsilon$. The concentration in moles (residue)/unit area per monolayer was taken from the concentration on the surface of the film balance at the moment of transfer. For collapsed films and monolayers where the transfer was not quantitative, the results are presented in terms of ΔA .

The net (left-right) linear component of the CD instrument was detected by interposing a Rochon prism in the light beam emerging from the stress modulator (18).

Maximum differential absorption signals were obtained when the Rochon prism was rotated to bring the plane of polarization of the ordinary ray to 45° clockwise (+45°) and 45° counterclockwise (-45°) to the perpendicular (Y axis) of the instrument. The extraordinary ray from the prism was blocked. A null signal was obtained when the plane of polarization of the ordinary ray was colinear with the Y axis.

RESULTS

The results of the pressure-area (π - A) and surface potential (ΔV) determinations on PMG and PA monolayers formed by spreading from chloroform-DCA solvent and PMG monolayers formed by spreading from pyridine-chloroform are shown in Fig. 1. Identical isotherms and spectroscopic results were obtained with pyridine-chloroform (98:2) and pyridine-chloroform-DCA (97:2:1) solvents, but dissolution of the polymer was easier in the latter system. Monolayers of PA and PMG formed from chloroform-DCA solvent exhibited a sharp rise in the surface pressure at about 14 and 16 Å²/residue, respectively, with a plateau in the PMG isotherm at about 20 mN/m and an inflection between 21 and 22 mN/m in the PA isotherm, in agreement with previous work (6, 7). A second type of isotherm was exhibited by PMG monolayers formed from 98% pyridine-2% chloroform spreading solvent which showed a smooth increase in surface pressure as the film area was decreased below 10-12 Å²/residue. The surface potentials of PA and PMG monolayers formed from chloroform-DCA spreading solvent are in agreement with values previously reported for both neutral and acid substrates (6, 7). The ultraviolet spectra of PMG monolayers transferred to quartz plates are shown in Fig. 2. For PMG films spread from chloroform-DCA solvent and transferred below the plateau pressure, the deposition appeared to be quantitative. In six separate runs,

PMG monolayers formed by spreading from chloroform-DCA and transferred at 15 mN/m gave a transfer ratio of 0.99 with a standard deviation of 0.07. The ratios were much less than unity when these films were transferred above the plateau pressure of 20 mN/m. Films formed from spreading solvents very high in pyridine content also gave low transfer ratios. Although not all films were transferred quantitatively as suggested by the low ratios, features such as the wavelength of the absorption maximum or the presence of the shoulder at 206 nm in the UV spectra of films formed from chloroform-DCA solvent were independent of transfer pressure. The Langmuir-Blodgett monolayers exhibited little if any light scattering with UV absorbances of less than 0.01 unit at 400 nm. The collapsed films exhibited some scatter with absorbances between 0.05 and 0.1 unit at 400 nm.

The linear dichroism (LD) of oriented films is shown in Figs. 3 and 4. Formation of a monolayer at the air-water interface by spreading from chloroform-DCA solvent followed by transfer at 15 mN/m pressure

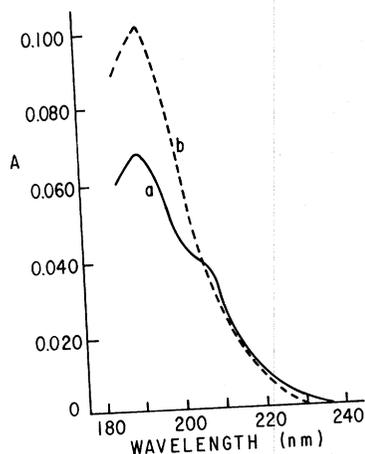


FIG. 2. Ultraviolet spectra of polypeptide monolayers. (a) Sixteen monolayers of polymethylglutamate spread from chloroform-dichloroacetic acid (99:1 vol); (b) polymethylglutamate monolayers spread from pyridine-chloroform (98:2). Both films transferred at 15 mN/m.

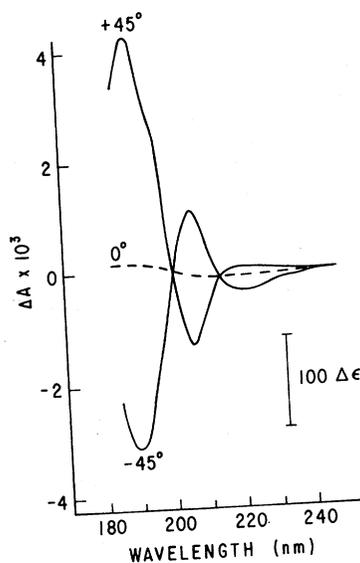


FIG. 3. Linear dichroism of sixteen monolayers of polymethylglutamate in the α -helical conformation. All eight plates oriented in the directions indicated. Single scan for each orientation.

to 8 quartz plates gave 16 monolayers which exhibited the spectra shown in Fig. 3. The plates were mounted in holder No. 1 and oriented in the light beam of the spectropolarimeter at various angles from the perpendicular as indicated. The spectra shown in Fig. 4 were obtained on a film prepared by collapsing a monolayer onto a single quartz plate. The monolayer had originally been prepared by spreading PMG at the air-water interface from pyridine-chloroform (98:2) solvent. The orientations indicated in Figs. 3 and 4 are accurate to $\pm 5^\circ$. Interpretation of the LD spectra is illustrated schematically in Fig. 5, which shows the interaction of oriented films with the (idealized) linear and circularly polarized light of the CD spectropolarimeter. Elimination of the linear component in the spectrum of PMG monolayers gave the true CD spectrum shown in Fig. 6 in which a raw spectrum and base line are presented. Separate experiments showed that almost an order of magnitude reduction in the linear dichroism evident in Fig. 3 could

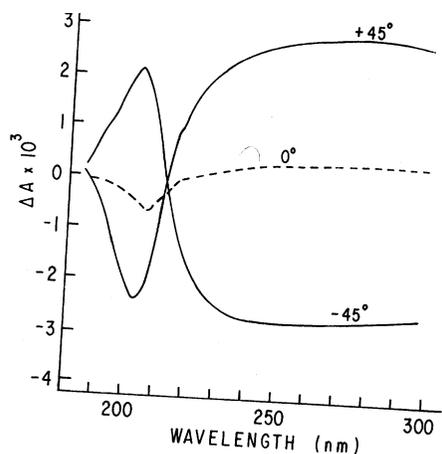


FIG. 4. Linear dichroism of a collapsed film of polymethylglutamate containing the β -sheet structure. Single scan for each orientation.

be effected by mounting the plates in only two orientations, 90° from each other. Increasing the number of orientations to four effected a further reduction in the linear component to the point where the characteristic features of the spectrum of polypeptides in the α -helical conformation were recognizable, but the shape of the CD curve was somewhat dependent on the particular placement of the sample in the light beam. With eight or more orientations of the plates, identical spectra were obtained which were

not dependent on the particular locations chosen for the plates. Sixteen orientations were used to obtain the spectrum shown in Fig. 6. All other spectra reported here were taken with eight orientations of the plates. The raw spectrum in Fig. 6 has been replotted in Fig. 7 together with the spectrum of a collapsed film of α -helical PMG. The spectra of monolayers and a collapsed film of PMG spread from pyridine-chloroform-DCA are shown in Fig. 8. The vertical bars represent the average peak-to-peak noise levels at various wavelengths in the original spectra.

The linear dichroism of a collapsed film of PA is shown in Fig. 9 and the CD spectra of the collapsed film and Langmuir-Blodgett monolayers of PA are given in Fig. 10. The monolayers were transferred at 15 mN/m.

DISCUSSION

General

The poly- γ -methyl-L-glutamate (PMG)-chloroform-pyridine system provided a means of preparing polypeptide monolayers with a high content of either α -helix or β -conformation. Poly-L-alanine (PA) was chosen as a second polypeptide for study

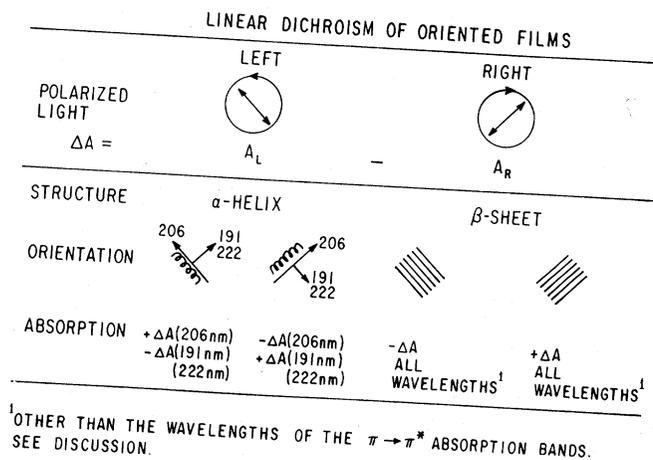


FIG. 5. Idealized illustration of the interaction of ordered films with the linear and circularly polarized light of a commercial spectropolarimeter.

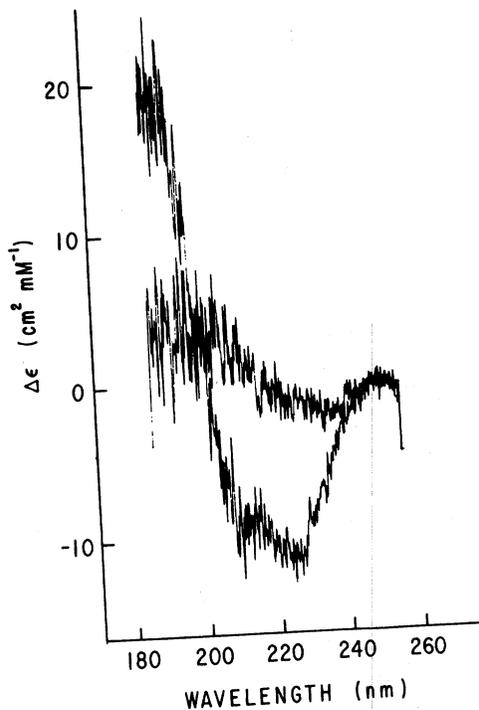


FIG. 6. Raw circular dichroism spectrum of the sixteen monolayers of polymethylglutamate used to obtain the linear dichroism spectra in Fig. 3. Thirty-two scans each for the base line and signal.

because its methyl side group is both hydrophobic and compact, contrasting with the bulky, relatively polar methyl glutamate group of PMG.

Monolayers of both polypeptides have been found to be well-ordered films when removed from the air-water interface (6, 7) which suggested potential problems in determining their true circular dichroism (CD) spectra. Commercial CD instruments such as the Jasco Model J-41C, which are based on the method of Grosjean and Legrand, produce polarized light with both linear and elliptical as well as circular components and will not, in general, correctly record the CD spectrum of a specimen in which the absorbing optically active molecules have a preferred spatial orientation (19, 20). Linear dichroism (LD) is much stronger than CD and the apparent CD of an oriented sample

will vary as it is rotated in the light beam of the spectropolarimeter (20). Linear dichroism can be quite useful, however, in studying the orientation of the molecules in a sample, and instruments have been specifically designed (21, 22) or modified (23) for the purpose. An unmodified commercial spectropolarimeter has been used to study electrically oriented molecules in solution (24) in a manner analogous to the method presented here for studying films. Interpretation of the LD spectra of films is given below for each polypeptide.

To determine the true CD of an oriented sample, the linear component must be eliminated; several suggestions have been made (20, 25, 26). Norden has suggested the summing of spectra taken with many orientations of the sample (25). Tunis-Schneider has shown on theoretical grounds that for either very thin or partially ordered samples, the average of two apparent CD

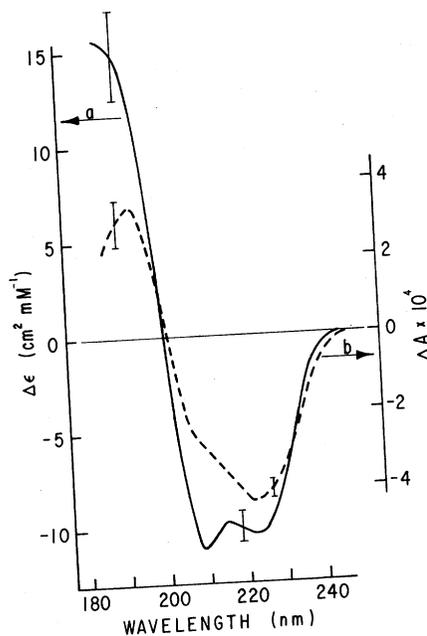


FIG. 7. Circular dichroism spectra of polymethylglutamate films. (a) Monolayers spread from chloroform-dichloroacetic acid (DCA) (99:1 vol); (b) collapsed film spread from chloroform-DCA (99:1).

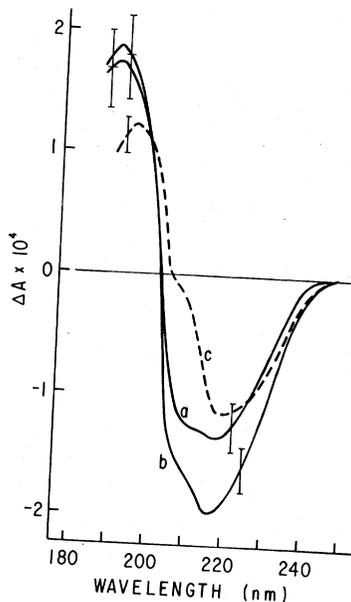


FIG. 8. Circular dichroism spectra of polymethylglutamate films. Monolayers spread from pyridine-chloroform-dichloroacetic acid (97:2:1 vol) and transferred at (a) 15 mN/m; (b) 22 mN/m; (c) collapsed film spread from pyridine-chloroform (98:2).

curves, taken with the sample at two orientations 90° apart around the axis of the incident light beam, should yield the true CD spectrum free of linear component (20). In practice the average CD spectrum of nucleic acid films was found to vary somewhat

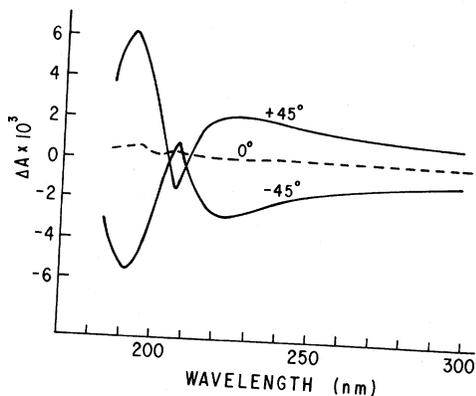


FIG. 9. Linear dichroism of a collapsed film of polyalanine spread from chloroform-dichloroacetic acid (99:1 vol).

with the particular orientation of the orthogonal pairs (20), which has also been noted in the present work on polypeptide films.

With eight orientations of the plates and 90° rotation of the sample at the mid-point in the data acquisition step, superimposable spectra were obtained, independent of the choice of locations for the plates (0, 45, 90°, . . . , vs 22½, 67½, 112½°, . . . , for example). This was taken to mean that the linear component had been minimized and was estimated to be at most 10% of the CD signal for films of PMG in the α -helical or β -conformation. The use of plate holder 2 without rotation did not always serve to eliminate the linear component, presumably because of nonuniformities between the orthogonally mounted pairs. Ninety degree rotation caused each plate to become its own orthogonal partner. Rotation of holder 2 through 90° was not counted as increasing the number of orientations of the plates. Each plate was moved to a previously occupied position, generating no new locations.

The CD of oriented systems can also be affected by LD and birefringence which is *not* a function of the angle of rotation about the optical axis of the instrument

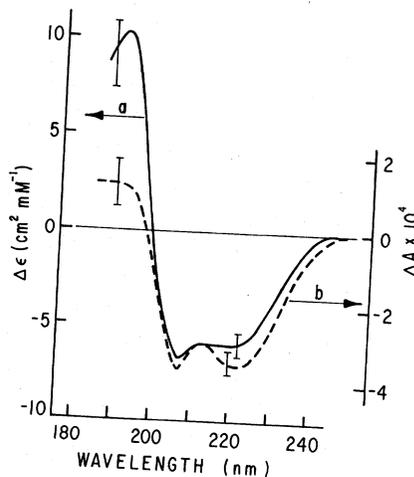


FIG. 10. Circular dichroism of (a) sixteen monolayers; (b) collapsed film of polyalanine. Spreading solvent, chloroform-dichloroacetic acid (99:1 vol).

(19). The main effect is underestimation of the apparent CD of the sample, but for a sample with LD ($A_y - A_x$) ≤ 0.5 , less than 10% error in the measured CD is to be expected (26). All samples reported here had $A < 0.2$, hence errors from this source should be negligible.

PMG Monolayers

The ultraviolet spectra of transferred PMG films shown in Fig. 2 are characteristic of polypeptides in the α -helical and β -sheet conformations (27) and depend on the spreading solvent used to form the monolayer. PMG monolayers formed from chloroform-dichloroacetic acid (DCA) gave a UV spectrum with maximum absorption at 191 nm and a shoulder at about 206 nm, characteristic of the α -helical conformation. Monolayers of PMG formed from pyridine-chloroform (98:2) gave a UV spectrum similar to that attributed to polypeptides in the β -conformation, with a maximum absorption at 192–193 nm. Infrared spectroscopy (8) and the CD spectra obtained in the present study indicate that PMG films formed from pyridine solvent contain a mixture of α -helical and β -sheet conformations. The absence of a shoulder in the 206- to 208-nm region of curve b, characteristic of the α -helix, suggests that the β -sheet conformation predominates in this film, consistent with the results of previous investigations (8). Transfer of the monolayers at 15 mN/m was effected at higher film concentration for the β -conformation than for the α -helix, but transfer ratios of 0.7 and below suggest that removal of the film from the air-water interface was not quantitative for the monolayers with a high β -content. The UV and CD data together suggest that the film containing the β -structure probably has about the same concentration of polypeptide per cm^2 on the quartz plates as the films where the polymer is in the α -helical conformation. The β -structure has a higher molar absorptivity for the band near 190 nm

than the α -helix (27). Absorption by the β -structure will be further enhanced in films mounted perpendicular to the light beam where the parallel transition moment of the β -sheet (12) is more favorably oriented with respect to the electric field vector of the incident light than the perpendicular transition moment for the α -helix (28).

In previous investigations, monolayers of polypeptides in the α -helical conformation were found to be well-ordered structures consisting of molecules with the helix axis oriented parallel to the compression barrier, normal to the sides of the trough (6, 7). This means that such films when transferred to quartz plates partially immersed in the subphase with their faces parallel to the sides of the trough, should have the helix axis of the polypeptides oriented "up" and "down" the plates as they are raised through the air-water interface. The interpretation of the LD spectra in Fig. 3 is consistent with this picture. Consider the plates oriented 45° clockwise from the perpendicular (+45°), as illustrated in Fig. 5. The absorption transition at 206 nm, which is parallel to the helix axis (28), is colinear with and will partially absorb the linear component associated with the right circularly polarized light, with minimal absorption of the linear component of the left circularly polarized light. According to the equation given in Fig. 5, this will produce a negative differential absorption band at about 206 nm. The absorption transition at 191 nm is normal to the helix axis (28) and will partially absorb the linear component of the left circularly polarized light, producing a positive differential absorption band near 191 nm. Repositioning the film at -45° will cause the recorded LD spectrum to be inverted compared to the spectrum recorded with the film at +45°. This is also shown in Fig. 3.

Transfer of the monolayer to quartz plates positioned in the film balance with their faces parallel to the compression barrier gave a film with the helix axes arranged in a hori-

zontal orientation, along the X axis of the quartz plates. This shows that the vertical orientation of the helices on the plates used to obtain the spectra in Fig. 3 was not due to alignment caused by raising the plates through the air-water interface. The ordering of the molecules has been ascribed to viscous drag in the film (7) but influences such as ripples on the surface caused by the moving barrier cannot be ruled out as possible contributing factors.

Collapsed films with the polypeptide collected as a multilayer on a single quartz plate gave LD spectra similar to those given in Fig. 3, showing that the collapse process does not destroy information about the orientation of the molecules in the monolayer.

Polarized UV spectra were determined on collapsed PMG monolayers in the α -helical conformation in a manner similar to that described by Gratzer *et al.* (28). The UV spectra (not shown) were similar to those previously reported (28) and consistent with the picture of α -helices oriented at the air-water interface of the film balance with the helix axis parallel to the compression barrier as found from the LD spectra.

LD spectroscopy can also give information about molecular orientation in films where the polypeptides are in the β -conformation. In this case the oriented polypeptide exhibits dichroism due to the parallel alignment of the adjacent, extended polymer chains which act as a polarizer, as well as dichroism due to the oriented absorption transitions. Consider a film of polypeptides in the β -conformation with the polymer chains oriented at $+45^\circ$ in the light beam of the spectropolarimeter as shown schematically in Fig. 5. This model suggests that the bulky methyl glutamate side groups are arranged so as to form part of the linear array, for example, alternately up and down along the chain. The polarizing action of the β -sheet will block the linear component of the left circularly polarized light, giving rise to a positive signal at all wavelengths outside of the band where the molecule ex-

hibits strong electronic absorption, as shown by the spectra in Fig. 4. The negative band centered at 203 nm in the recorded spectrum is undoubtedly the result of overlap from the bands of the β -sheet and α -helix portions of the mixed film. The polarization of the $\pi \rightarrow \pi^*$ transition in β -sheets parallel to the polypeptide backbone (12) will result in a negative differential absorption in the 200-nm region for films with the polymer axis oriented at $+45^\circ$. If the reasonable assumption is made that the axes of the helical portions of this film are colinear with the β -chains, then one can predict that the α -helix will contribute a positive band at about 191 nm and a negative band at about 206 nm to the recorded LD spectrum. Overlap of the bands due to the α -helix and β -conformation in a film where the β -structure predominates could well result in a LD spectrum as shown in Fig. 4, with the negative band at about 203 nm located at higher wavelength than would be expected for a pure β -sheet. It is probable that the true position for the $\pi \rightarrow \pi^*$ absorption transition of PMG in the β -conformation is given by neither the absorption nor the LD spectra shown in Figs. 2 and 4, respectively. The much weaker $n \rightarrow \pi^*$ transitions occurring over the 215- to 225-nm region for the α -helix and the β -conformation are not evident in the LD spectra of Fig. 4. The LD spectra of PMG monolayers formed at the air-water interface from 2% chloroform in pyridine spreading solvent is thus consistent with the picture of a film which is a mixture of the α -helix and β -sheet conformations where the helices and β chains are colinear and parallel to the compression barrier.

Although the CD spectrum of PMG monolayers in Fig. 6 is qualitatively that of a polypeptide in the α -helical conformation, it differs in detail from the spectra of solutions of homopolypeptides, in which the polymers are assumed to be pure helices. The negative bands at 222 and 208 nm, the crossover near 200 nm, and the positive

band near 190 nm are all features characteristic of polypeptides in the α -helical conformation (3). Inspection of the spectrum replotted as curve (a) in Fig. 7 shows, however, that the band at 190 nm is less intense by 6–8 $\Delta\epsilon$ units than that observed for solutions of PMG in the α -helical conformation (15), and the crossover at 199–200 nm seems to occur at slightly shorter wavelength than is usually associated with the pure α -helix (15, 16, 29, 30). Although the noise level is high, the spectrum shown in Fig. 6 is reproducible, and the features mentioned above have persisted in all runs with monolayers of PMG in the α -helical conformation. Comparison of the spectrum (a) of Fig. 7 with the computed spectra of poly-L-lysine in the α , β , and random conformations (29) suggests that the monolayers of PMG may contain some disordered structure. The $\Delta\epsilon$ of 15 at 190 nm and the crossover at 199–200 nm suggest 20% random conformation. However, the planar mounting of the polypeptide monolayers suggests an alternate explanation for the deviation of the CD spectrum from that normally found with isotropic solutions. Polypeptide monolayers confined to a plane with the helix axis perpendicular to the propagation vector of light would be expected to give a CD spectrum with little or no contribution from the light beam travelling along the helix, colinear with the axis. The axial component has been determined on hexafluoroisopropanol solutions of electrically oriented PMG and was found to have the shape of the first derivative of the perpendicular $\pi \rightarrow \pi^*$ (190 nm) band (15). Adding the axial component back to Fig. 7a increases the 190 band by about 6–8 $\Delta\epsilon$ units and shifts the crossover to longer wavelength, by about 2 nm, giving a CD spectrum more nearly characteristic of isotropic solutions of PMG. Although the axial component is somewhat solvent dependent (15), the comparison does suggest that deviation of the PMG monolayer spectrum from "ideal" may be due in part to

incidence of the light beam normal to the helix axis. The above discussion suggests that the CD spectrum of PMG monolayers shown in Fig. 6 is that of a polypeptide largely in the α -helical conformation, possibly containing a small amount of disordered structure. This is consistent with the results of previous investigations (6–8).

The CD spectra of PMG monolayers transferred at 28–30 mN/m (not given), which is above the plateau pressure of 20 mN/m shown in Fig. 1a, exhibited the features characteristic of the α -helix. This is in agreement with the results of previous investigations (8) and consistent with the suggestion that the plateau results from a monolayer to bilayer transition of α -helical polypeptides rather than a conformation change during compression (7).

In contrast to the α -helical conformation, for which theoretical and experimental CD work is usually performed on isotropic solutions, the CD of the β -structure has frequently been studied in films (10–12, 31). The similarity between the CD spectra of films and solutions of poly-L-lysine in the β -conformation (10) suggests that the CD transitions found in solutions over the observable UV range are also present in films. Consistent with this are theoretical calculations which have shown that for the β -pleated sheet all absorption bands above 180 nm are expected to have transition moments either entirely or largely in plane (31). Only the relatively weak absorption transition at 175 nm in the antiparallel sheet has a moment perpendicular to the plane (31).

The CD of PMG monolayers formed from pyridine–chloroform–DCA (97:2:1 vol) solvent gives clear evidence of both the β -sheet and α -helix conformations. The negative bands at about 217 and 208 nm are characteristic of the β - and α -structures, respectively. The crossover at 202 nm is shifted to slightly longer wavelengths from that observed for PMG monolayers in which the polypeptide is almost pure α -helix. This suggests the presence of β -structure in

the film, since the β -conformation exhibits a crossover at 206–207 nm (10, 29, 32). The UV, LD, and CD data for this film suggest that it is a mixture of α -helix and β -sheet in which the β -conformation predominates. This agrees with previous investigations employing infrared spectroscopy in which it was shown that PMG films with a high β content always contained some polypeptide in the α -helix conformation, even when pure pyridine was used to form the monolayer (8).

The CD of the collapsed films of PMG monolayers formed from chloroform–DCA and pyridine–chloroform solvents show the same general features as the spectra of the corresponding Langmuir–Blodgett monolayers, but with some differences probably attributable to light scattering. Urry *et al.* (11, 30) showed that light scattering by suspensions and films of polypeptides in the α -helical conformation caused a decrease in all CD bands which became especially pronounced at shorter wavelengths. A shift in the crossovers to longer wavelengths was also observed. The decrease in the intensity of the bands at 208 and 190 nm and the shift of the crossovers to longer wavelength in the spectra of collapsed films as compared to the spectra of the corresponding monolayers can be seen in Figs. 7 and 8. Disordered structure can also cause a decrease in the intensity of the bands below 210 nm in the spectra of the α -helix and β -conformations, but the crossovers in the spectra of the mixtures will remain unchanged or be shifted to shorter wavelengths compared to the spectra of pure α or β (29). Clearly this is not the case with the spectra presented in Figs. 7 and 8. The spectra of both the collapsed films and the Langmuir–Blodgett monolayers thus suggest that the PMG films are almost pure α -helix when the monolayers are spread from chloroform–DCA and that mixtures of α -helix and β -sheet result when pyridine–chloroform is the spreading solvent. This is consistent with the results of

previous investigations using infrared spectroscopy which showed that gross structural changes were not introduced by the collapse process (8).

PA Monolayers

The CD and linear spectra of PA monolayers are consistent with a picture of polypeptides in the α -helical conformation, oriented at the surface of the film balance with the helix axes parallel to the compression barrier. Collapsed films of PA exhibited dichroism due to orientation of the absorption transitions of the α -helix and dichroism due to orientation of the helix itself, which acted as a polarizer as shown in Fig. 9. The interpretation of the LD spectra follows arguments similar to those for PMG films as outlined in Fig. 5.

It is interesting to note that for oriented monolayers of polypeptides in the α -helical conformation, PA exhibited linear dichroism due to polarization but PMG did not. Space-filling models of PA show that this polypeptide is a compact chain in the α -helical conformation capable of forming a well-ordered linear array which can act as a polarizing element. Replacement of the methyl side group of PA with the bulky methyl glutamate side chain of PMG can give a structure in which the polarizing pattern is disrupted even though the helix is oriented and capable of exhibiting dichroism due to the absorption transitions.

The CD spectrum of Langmuir–Blodgett monolayers of PA, in Fig. 10, is similar to those of α -helical polypeptides, though qualitative and quantitative differences are observed. The negative bands are of lower intensity than is usually observed and the band at 206–207 nm is at slightly shorter wavelengths than the 208 nm position previously recorded on solutions of this polymer (3, 11, 16). Parrish and Blout (16) reported a spectrum of PA in highly purified hexafluoroisopropanol similar to that shown in Fig. 10a, but with even larger deviations in

band position and intensity. They ascribed the spectrum to an α -helix distorted by hydrogen bond interaction with the solvent.

The role of the axial component in the CD spectrum of this polymer is not clear. The intensity ratio, 190 band:206 band, is nearly the same for films (Fig. 10a) and solutions of PA in highly purified hexafluoroisopropanol (16), but addition of water to the solution caused the CD spectrum of PA in solvent to return to "normal" with an increase in the relative intensity of the 190 nm band (16).

The CD spectrum of a collapsed film of PA in Fig. 10b shows a severe flattening in the short wavelength band and a crossover at about 197 nm, about 2 nm below the crossover in the monolayer spectrum. The light scattering in this film, observed as an absorbance of about 0.1 unit at 400 nm, probably caused much of the decrease in the 190 nm band (11), but the position of the crossover at shorter wavelength than in the spectrum of the Langmuir-Blodgett monolayer, Fig. 10a, suggests that disordered structure may be partly responsible.

CONCLUSIONS

Despite the use of oriented films in a highly concentrated state, mounted in a planar configuration and surrounded by a gaseous medium, the resultant CD spectra are qualitatively quite similar to spectra normally associated with polypeptides in the α -helix and β -sheet conformations in solution. Thus, once mounted on quartz plates, PMG and PA monolayers yield CD spectra that can be interpreted in terms of familiar structures.

While comparable information about the conformation of biopolymers is obtainable from either IR or CD spectroscopy, an advantage of CD is the ability to study hydrated structures without the need for using D₂O media (9). This is an important benefit in studying surfaces of biochemical interest since differences in protein conformation in wet and dry films have been observed (13).

ACKNOWLEDGMENTS

The author thanks R. R. Calhoun, G. Pisano, and J. Skasko, Plant Management, ERRC, for assistance in design and construction of the film balance and plate holders, and Dr. E. M. Brown of the Dairy Laboratory, ERRC, for many helpful discussions. Loan of the Rochon prism by J. Aviv of Aviv Associates, Lakewood, N. J., is gratefully acknowledged.

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