NRL Report 6554

Infrared Spectra of Surface Films of Poly-γ-Methyl-L-Glutamate

George Loeb and Robert E. Baier

Surface Chemistry Branch
Chemistry Division

July 20, 1967

NAVAL RESEARCH LABORATORY
Washington, D.C.

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

ABSTRACT

Surface pressure vs area isotherms of poly-γ-methyl-L-glutamate spread on water from solutions of chloroform differed from those obtained when the polymer was spread from solutions of high pyridine concentration. Samples for studying infrared spectra of the material forming the monolayer were obtained by collapsing the monolayer onto a transparent plate or by depositing a monolayer by the Blodgett technique onto a multiple internal reflection prism. When solutions of the polypeptide were spread from chloroform upon water in a Langmuir-Adam film balance to form an insoluble monolayer, a plateau in the force-area isotherm and a large limiting area were observed. Spectra of these monolayers were characterized by amide I and II regions characteristic of the α-helical or random configuration. There was not a change in secondary structure corresponding to the plateau region detectable via the IR spectra. Spreading solutions of high pyridine concentration led to isotherms without a plateau and with a much smaller limiting area. Spectra of these monolayers showed a significant amount of β-type material. This is the first unambiguous correlation between the infrared spectra of uncollapsed monolayers of polypeptides and their behavior at the air/water interface.

PROBLEM STATUS

This is an interim report; work on this problem is continuing.

AUTHORIZATION

NRL Problem C03-01
Project RR 007-08-44-5503

Manuscript submitted February 20, 1967.

INFRARED SPECTRA OF SURFACE FILMS OF POLY-γ-METHYL-L-GLUTAMATE

INTRODUCTION

The conformations of proteins and polypeptides in the solid and solution states have been successfully studied by means of their spectra. It was felt that infrared spectra could also yield information bearing on their conformations in surface films.

The surface pressure (π) vs the available area (A) per amino acid residue (or monomer) unit isotherms of a number of polypeptides have been studied by Isemura and coworkers. In a study of a series of uncharged synthetic polypeptides having various paraffinic side-chains, Isemura and Hamaguchi (1952, 1953) found that those polymers with long side-chains produced π vs A curves consisting of three regions: as the polymer film was compressed from very high values of A, a steep rise in π occurred; then, at a well-defined surface pressure, a plateau in the π vs A diagram was reached; no further increase in surface pressure was registered until compression had continued to a considerably lower value of A. In the case of poly-α-amino lauric acid, for example, the plateau region extended from 22 A^2/res to 15 A^2/res. A plateau was also found in the case of poly-γ-benzyl-L-glutamate. The case of poly-γ-methyl-L-glutamate (PMLG), however, was quite different. The limiting area (A_0) proved to be only 10 A^2 per monomer (or per residue) instead of the value expected for a fully spread polypeptide (i.e., $\geq$15 A^2/res). On the surface of acidic water they found that A_0 became much larger. No plateau was found in either case for PMLG.

Isemura and Hamaguchi assign the force-area curve with low limiting area to the coiled α-form of PMLG and the higher-limiting-area curve to the β-form. The plateaus found for long-chain compounds were assumed to correspond to transitions between configurations with the side chains flat on the water surface and the more compact β-structure. On the other hand, Low (1953) and Bamford, Elliot, and Hanby (1956) postulated that the α-helix would have larger area than the β-type structure, while Crisp (1958) presented the viewpoint that a plateau could very well be attributed to a collapse type of phenomenon. Thus, although it is obvious that a transition process had occurred in the surface film, it was not at all clear what that process was.

Malcolm (1962) investigated monolayers of some polypeptides spread on water. He took infrared spectra of collapsed films derived from spread monolayers and also measured the deuterium-hydrogen exchange process in the spread monolayers. He concluded that the polypeptides existed in the α-helical configuration at the surface when spread, as the amide infrared bands were found in the positions corresponding to α-structures and also because the deuterium-hydrogen exchange was quite slow. He also postulated that with increased time on the surface at high pH and raised temperature the PMLG, initially α, became converted to the β-configuration, since he found a faster isotopic exchange rate and a different spectrum. He did not find a plateau in the π vs A curve of PMLG. These results, although of interest, are limited by two considerations: first, only spectra of collapsed specimens were taken, and second, it has since been recognized (Schellman and Schellman (1964)) that deuterium-hydrogen exchange is complicated by the effect of the environment of the exchanging groups, and the effect of a nearby surface on exchange has not yet been independently investigated.

The uncertainties of conformation in these polypeptide films suggested a reinvestigation of the infrared spectrum of PMLG monolayers. To try to avoid possible effects of collapse upon the spectra of the surface films, we obtained samples for spectral studies by the Langmuir-Blodgett deposition technique, for films spread from both chloroform and pyridine-rich spreading solutions, at surface pressures above and below the pressure at which a plateau was found. Multiple internal reflection (MIR) spectra could be obtained from these samples. For comparison we also obtained spectra from collapsed monolayers.

EXPERIMENTAL PROCEDURE

The PMLG used was a gift from Dr. Reeder of Courtaulds, Ltd. It was dried under vacuum before use and stored in the refrigerator.

Pyridine of reagent grade was distilled from an all-Pyrex still before use. Chloroform of reagent grade was purified by shaking with sulfuric acid, sodium hydroxide, and water, as suggested by Hanlon and Klotz (1965), then dried with Drierite before distilling from a Pyrex still. Results obtained with this treatment were the same as were obtained with reagent grade chloroform used without further purification.

The polymer was spread with each of these solvents (or mixtures thereof) using solutions of 0.2-0.7 mg/ml concentration. As the polymer sample was not completely soluble in pyridine, solutions were made up (by weight) by dissolving PMLG in a small amount of chloroform, then slowly adding the proper amount of pyridine with gentle stirring. All solutions were stored in the dark until used.

Water was obtained from a tin-lined still. A few experiments using triply distilled water from a quartz still yielded essentially similar results.

The water surface was tested for contamination, by compression upon the Langmuir trough, before each isotherm was obtained.

Monolayers were spread on the water subphase with a Hamilton syringe by using a Misco micrometer device for delivery and a Teflon needle. A droplet was formed at the needle tip and touched to the water surface. The nonwetting Teflon needle allowed the high-pyridine-content solutions to be spread more easily.

Isotherms of π vs A were obtained in an air-conditioned room kept at $21 \pm 1°C$. A completely enclosed Pyrex trough, with waxed edges, was fitted with a Cenco torsion head, phosphor bronze torsion wire, and a waxed mica float. The trough dimensions were $60 \times 15 \times 0.3$ cm. Sensitivity of 0.3 dyne/cm/degree and precision of ± 0.1 degree were achieved. Experiments indicated that a 15-minute interval and a 30-minute interval between spreading of the film and compression led to essentially the same π vs A isotherms, therefore a 20-minute interval was used routinely.

The π vs A isotherms were produced quite slowly: at high pressures it sometimes took 10 minutes for the surface pressure to decay to a steady value after each area decrement; film rigidity made very precise adjustment difficult. The surface pressure was recorded after it remained sensibly constant for 2 minutes.

Samples for spectral analysis were obtained in two ways:

1. A clean germanium multiple-internal-reflection prism was immersed in a water-filled Pyrex trough deeper than the one used to obtain the isotherms. The plates, $20 \times 50 \times 1$ mm, obtained from Wilks Scientific Corp., were hung with their large faces vertical and parallel to the barrier. A monolayer was spread, compressed to the desired

pressure, and maintained at that pressure while the germanium plate was drawn slowly upward, through the monolayer, at a rate of 1 mm/min. At this rate, drainage of the plate allowed the monolayer to adhere without a visible underlying layer of water. The monolayer was maintained at constant pressure by the method of Sher and Chanley (1954), using a Teflon float and a lightly waxed silk thread to contain the film. The germanium plates were then mounted in a Wilks Scientific Corp. single-beam multiple-internal-reflection accessory, with angle of incidence of 45°, for obtaining spectra.

2. Collapsed monolayers were obtained by the method of Malcolm (1962). After a π vs A isotherm was taken, the monolayer was compressed until only a 5-mm width was left between barriers. A 5 ×15-mm strip of silver chloride was immersed half way and passed the width of the trough, between the barriers. The collapsed layer formed a narrow stripe on the silver chloride plate, which was then held in a beam condenser assembly in the spectrometer.

All spectra were obtained on a Beckman IR 12 spectrometer.

The procedure for cleaning the germanium plates must remove previous monolayers and leave a uniform thin layer of liquid on the surface, without ruining the transmission in the infrared. After some experimentation, the procedure adopted was to wash the plate with the detergent Tide, rinse it with water and acetone, dry it, and expose it to cathodic bombardment at 2 kv under 30 microns residual air pressure for 5 minutes. The resulting surface which when tested with water was well wetted, drained so uniformly that interference fringes of water draining from a clean plate were horizontal and parallel. Plates were cleaned just before use, were wet with clean water to check their drainage, and were immediately hung in the trough before the monolayer was spread.

Optical rotatory dispersion (ORD) measurements were performed on two spreading solutions: PMLG in 100% chloroform, and PMLG in 7% chloroform-93% pyridine. A Cary model-60 spectropolarimeter was made available by Dr. E. Charney, of the National Institutes of Health, for these measurements. ORD data were plotted according to the Moffitt equation (see Urnes and Doty (1961)) and b_0 values determined.

Small portions of these spreading solutions were also allowed to evaporate to dryness on silver chloride strips, and spectra were taken in a manner similar to that used for the collapsed monolayers.

RESULTS

Curves of π vs A for PMLG spread on water from high concentrations of pyridine (>85%) were similar to those reported by Isemura and Hamaguchi (Fig. 1, curve a). They showed no plateau, and there was a low limiting area varying from 10 to 11 A^2/residue, depending on the part of the sample of polymer used. This may be compared with the value of 10 A^2/residue reported by Isemura and Hamaguchi. When spread from a spreading solution of chloroform concentration greater than 60%, however, a much different type of π vs A curve was obtained. After an initial rise to 19 dynes/cm, a long plateau was produced, similar to those found by Isemura and Hamaguchi for other polypeptides with long side-chains. After compression to about one-half the limiting area, the isotherms again began to rise steeply as compression was continued. The limiting area of this isotherm was 16-17 A^2/residue, again depending upon the part of the sample used (Fig. 1, curve c).

Due to the limitations of the apparatus the surface pressure could not be followed above 35 dynes/cm; however, in the range above 20 dynes/cm the films produced from high chloroform and high pyridine concentration spreading solutions were very similar in the sense that the surface pressure increased linearly with decreasing available area.

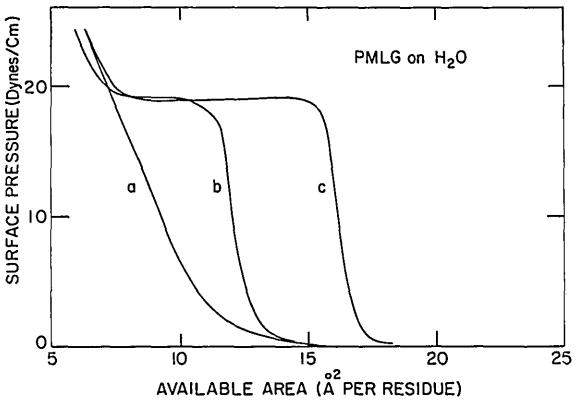
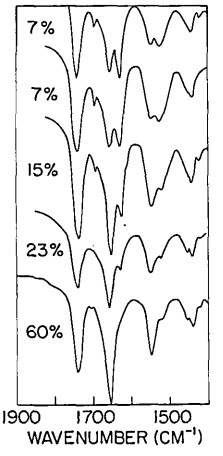


Fig. 1 - Force-area isotherms of poly-γ-methyl-L-glutamate spread from spreading solutions of varying chloroform/pyridine content.

Spreading solvents of intermediate composition yielded isotherms of intermediate character, as shown in Fig. 1, curve b, which was produced by a spreading solution of 23% chloroform. Isotherms of chloroform concentration greater than 60%, and smaller than 15%, yielded isotherms indistinguishable from those shown in Fig. 1, curves c and a, respectively.

When the monolayers from a series of spreading solutions of varying chloroform/pyridine ratios were collapsed and spectra taken, the results shown in Fig. 2 were obtained. Comparison of Fig. 2 with Fig. 1 shows that the nature of the isotherm could be correlated with the spectrum. A curve with a long plateau region corresponded to a single absorbtion peak in the amide I region at 1654 cm^{-1}, while loss of the plateau was accompanied by appearance of a second absorbtion at 1630 cm^{-1} at the expense of some absorbtion at 1654 cm^{-1}. A similar correlation is found in the amide II region, at 1550 and 1530 cm^{-1}, respectively.

Fig. 2 - Infrared transmission of collapsed films of PMLG spread from spreading solutions of varying chloroform/pyridine content.



MIR spectra of the deposited monolayers are shown in Fig. 3, for the case of 100% chloroform spreading solutions at surface pressures below and above the plateau pressure, and in Fig. 4 for 93% pyridine spreading solution. These are shown on expanded scale in Fig. 5. It will be noticed that in these spectra, as in the collapsed monolayer spectra, monolayers from high chloroform concentration spreading solutions show a

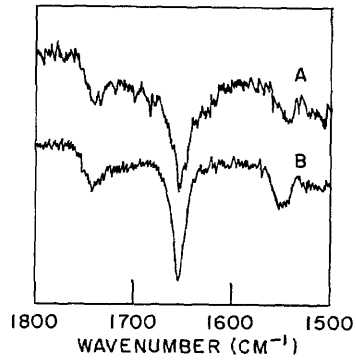


Fig. 3 - Infrared transmission of deposited monolayers derived from chloroform spreading solvent: Curve A -- Deposited at π = 4.2 dynes/cm; Curve B -- Deposited at π = 26.5 dynes/cm.

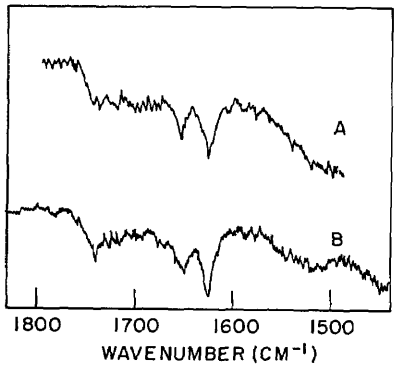


Fig. 4 - Infrared transmission of deposited monolayers derived from 93% pyridine spreading solvent: Curve A -- Deposited at π = 15 dynes/cm; Curve B -- Deposited at π = 27 dynes/cm.

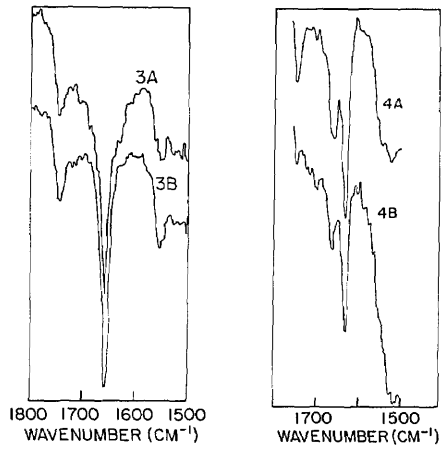


Fig. 5 - Expanded scale displays of the spectra shown in Figs. 3 and 4.

large amide I band at 1654 cm^{-1} whereas those from high pyridine show two bands, at 1630 and 1654 cm^{-1}. A steep background prevents critical analysis of the amide II region at 1530-1550 cm^{-1}.

Exact correspondence of band ratios in collapsed and monolayer samples should not be expected, even if the same ratios of material absorbing at 1654 and 1632 cm^{-1} are present, since polarization of the infrared radiation is different with respect to possible orientations of the sample. The amide II region from collapsed spectra always showed a very small 1530 cm^{-1} component, even when the sample was spread from chloroform.

There was a small component at 1700 cm^{-1} which appeared to be correlated with the 1630 cm^{-1} component in the spectra from collapsed films.

Dried-down samples of both chloroform and pyridine-rich solutions (Fig. 6) showed similar spectra and were characteristic of samples spread from chloroform rather than from pyridine-rich solutions.

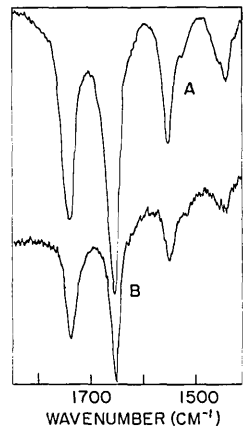


Fig. 6 - Infrared transmission of spreading solutions, evaporated to dryness: A -- chloroform solution; B -- 93% pyridine solution.

The optical rotatory dispersion data, when plotted according to the Moffitt equation (Urnes and Doty (1961)) yielded values of the b_0 parameter of -325 ± 25 for the pyridine-rich, and -545 ± 10 for the chloroform, spreading solutions.

DISCUSSION

The amide I (1650 cm^{-1}) and amide II (1550 cm^{-1}) regions of polypeptide spectra are thought to be indicative of the secondary structure of the polymer. Strong absorption at 1650 to 1660 cm^{-1} is characteristic of random-chain or α-helical structures, while absorption at 1630 cm^{-1} is characteristic of β-type structures (see, for example, Miyazawa (1963)). A similar relationship holds for the amide II region: 1550 cm^{-1} is related to the α, and 1530 cm^{-1} to the β structures.

A small peak at 1700 cm^{-1} may be due to the antiparallel β-conformation, since other polypeptides in this form show similar absorption in the range 1685-1700 cm^{-1} (Bamford et al (1956), Schellman and Schellman (1964)).

One cannot very well distinguish between helical and random-chain structures by peak frequency alone in this spectral region; therefore, the fractions of random-chain and helical structures will not be known in these samples until studies of film orientation with polarized radiation are completed. However, we can detect changes to and from the β, or sheet, configurations by the techniques used here.

On this basis, our results indicate that isotherms of type a in Fig. 1 were produced by films which could form the β-structure to a large extent. In contrast, films whose isotherms were of type c, in the case of PMLG under these conditions, lead to overwhelmingly α-type spectra.

The spectra of films from pyridine-rich and chloroform spreading solutions showed different spectra when collapsed, whereas the spectra of monolayers whose isotherms

showed a plateau were similar whether picked up at pressures above or below the plateau pressure. In the light of these results, it is concluded that the process occurring in the plateau region does not cause the film to change to the form which results from pyridine-rich spreading solutions, despite convergence of the isotherms. One is also led to conclude that the polypeptide does not change in the manner characteristic of an α-β transition during the plateau, but rather that some other process occurs; perhaps an aggregation, or partial collapse, or conversion between random chain and helical forms of structure.

When samples of both spreading solutions were dried, the resulting spectra were both characteristic of α or random-chain structures. This result might lead one to believe that some phenomenon occurring during spreading causes the polymer from pyridine solution to revert to the β-form although it was α in the bulk solution. The ORD data, however, indicate that the polymer in pyridine-rich solution has a smaller helical content than the polymer in chloroform solution, since the b_0 parameters are -325 and -550, respectively. The nonhelical fraction of polymer may revert to the β-form, while the α-helical fraction's conformation is retained.

In chloroform, on the other hand, a much higher degree of helicity exists, and it is possible that the helix stability prevents the material from becoming β unless it is overcome by means other than spreading, such as heating and pH changes, as was indicated by Malcolm's work described above.

It should be noted that all the spectra were taken of dried samples. We have no way of knowing, at this stage, whether or not material in the aqueous surface is in the β-form, or becomes β upon drying; however, we have established that material from chloroform solution will not become β to any large extent, nor will material dried from pyridine by evaporation. Therefore, any β material must have been predisposed to such a condition by being spread, even if it was not in fact in the β form on the aqueous surface.

CONCLUSIONS

1. The combination of infrared spectroscopy, and in particular the newer MAIR (multiple attenuated internal reflection) techniques, with traditional monolayer methodology has allowed the unambiguous correlation of the backbone structure of polypeptides and their behavior at the air/water interface.

2. It has been demonstrated, for the first time in uncollapsed monolayer samples, that two important hydrogen-bonded polypeptide configurations are apparently stable in interfacial films. These configurations have infrared spectra characteristic of the intrachain hydrogen-bonded coil called the alpha-helix and the interchain hydrogen-bonded extended-chain configuration called the beta-structure.

3. It has been further demonstrated that the polypeptide spectra initially found upon surface spreading on water persists through compression of the films to high surface pressures and is also retained in collapsed skims from such spread films. In particular, contrary to previous interpretation of similar results, no backbone configuration change accompanies the plateau which characterizes force-area curves of poly-methyl-glutamate spread from 100% chloroform.

4. The fact that the spectral properties of this polymer do not change upon collapse will increase confidence in the relevance of structural studies of collapsed monolayers in other systems by techniques which require bulk amounts of material (such as x-ray diffraction and NMR spectroscopy).

ACKNOWLEDGMENT

We are grateful to Dr. R. E. Kagarise, previously of this Laboratory, and Miss D. S. Cain, of this Laboratory, for aid in obtaining the IR spectra, to Dr. E. Charney, of the National Institutes of Health, for making a Cary Model-60 Spectropolarimeter available to us, to Dr. N. J. Harrick, of Philips Laboratories, for discussions of the MATR technique, and to Mr. R. Olfky, of this laboratory, for aid in construction of the discharge cleaning apparatus.

REFERENCES

Bamford, C.H., Elliott, A., and Hanby, W.E. (1956), "Synthetic Polypeptides," New York: Academic, p. 355 et. seq.

Crisp, D.J. (1958), "Surface Films of Polymers" in "Surface Phenomena in Chemistry and Biology," Danielli, J.F., Pankhurst, K.G.A., and Riddiford, A.C., eds. New York: Pergammon, p. 23 et. seq.

Hanlon, S., and Klotz, I.M. (1965), Biochemistry, 4:37

Isemura, T., and Hamaguchi, K. (1952), Bull. Chem. Soc. Japan 25:40, 26:425 (1953)

Low, B. (1953) in "The Proteins," Neurath, H., and Bailey, K., eds., Vol. 1, Part A, New York:Academic, p. 235

Malcolm, B.R. (1962), Nature 195:901

Miyazawa, T. (1963), "Infrared Studies of the Conformations of Polypeptides and Proteins," in "Aspects of Protein Structure," Ramachandran, G.N., editor, New York: Academic, p. 257

Schellman, J.A., and Schellman, C. (1964), "The Conformation of Polypeptide Chains in Proteins," in "The Proteins," Neurath, H., editor, New York:Academic, p. 1

Sher, I.H., and Chanley, J.D. (1955), Rev. Sci. Instr. 26:266

Urnes, P., and Doty, P. (1961), Advan. Protein Chem., 16:401

GPO 927-633

UNCLASSIFIED
Security Classification

DOCUMENT CONTROL DATA - R & D
(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
Naval Research Laboratory
Washington

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP

3. REPORT TITLE
INFRARED SPECTRA OF SURFACE FILMS OF POLY-γ-METHYL-L-GLUTAMATE

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)
This is an interim report; work on this problem is continuing.

5. AUTHOR(S) (First name, middle initial, last name)
George Loeb and Robert E. Baier

6. REPORT DATE
July 20, 1967

7a. TOTAL NO. OF PAGES
11

7b. NO. OF REFS
10

8a. CONTRACT OR GRANT NO.
NRL Problem C03-01

b. PROJECT NO.
RR 007-08-44-5503

c.

d.

9a. ORIGINATOR'S REPORT NUMBER(S)
NRL Report 6554

9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)

10. DISTRIBUTION STATEMENT
Distribution of this report is unlimited.

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY
Department of the Navy (Office of Naval Research) Washington, D. C. 20360

13. ABSTRACT
Surface pressure vs area isotherms of poly-γ-methyl-L-glutamate spread on water from solutions of chloroform differed from those obtained when the polymer was spread from solutions of high pyridine concentration. Samples for studying infrared spectra of the material forming the monolayer were obtained by collapsing the monolayer onto a transparent plate or by depositing a monolayer by the Blodgett technique onto a multiple internal reflection prism. When solutions of the polypeptide were spread from chloroform upon water in a Langmuir-Adam film balance to form an insoluble monolayer, a plateau in the force-area isotherm and a large limiting area were observed. Spectra of these monolayers were characterized by amide I and II regions characteristic of the α-helical or random configuration. There was not a change in secondary structure corresponding to the plateau region detectable via the IR spectra. Spreading solutions of high pyridine concentration led to isotherms without a plateau and with a much smaller limiting area. Spectra of these monolayers showed a significant amount of β-type material. This is the first unambiguous correlation between the infrared spectra of uncollapsed monolayers of polypeptides and their behavior at the air/water interface.

DD FORM 1 NOV 65 1473 (PAGE 1)
S/N 0101-807-6801

88 a/b

UNCLASSIFIED
Security Classification

14. KEY WORDS	LINK A ROLE	LINK A WT	LINK B ROLE	LINK B WT	LINK C ROLE	LINK C WT
Infrared spectra						
Surface films						
Poly-γ-methyl-L-glutamate						
Surface chemistry						
Surface pressure-area isotherms						
Polypeptide configurations						
Multiple internal reflection spectra						
Optical rotatory dispersion						
Monolayers						
Polypeptide films						