The Influence of Polymer Conformation on the Surface Properties of Poly(γ-methyl L-glutamate) and Poly(benzyl glutamate)

Robert E. Baier[1a] and William A. Zisman[1b]

Laboratory for Chemical Physics, Naval Research Laboratory, Washington, D. C. 20390.
Received August 13, 1969

ABSTRACT: Reactions at protein surfaces are of immense importance in biology and technology, but are difficult to understand because of the complexity of protein molecules. In this investigation, a simple protein analog—the synthetic polypeptide, poly(γ-methyl L-glutamate)—was studied by internal reflection infrared spectroscopy and contact angle techniques. Thin polypeptide films with differing polymer backbone conformations were produced by treating poly(γ-methyl L-glutamate) film stock (about 4 mils thick) and solvent-cast thinner films with pure concentrated reagents such as formic acid, dichloroacetic acid, and chloroform. The wettability of these films proved to correlate well with structural assignments deduced from their infrared surface spectra. Coiled polymer configurations (*e.g.*, α-helix, random tangle) correlated with wetting behavior typical of an accessible polyamide backbone capable of hydrogen-bond interactions with contacting liquids. Extended-chain configuration (*i.e.*, the β structure) exhibited contact angle values indicative of only the methyl ester side chains in the outermost surface layer and no accessibility of H-bonding liquids to the polypeptide backbone. Since structural transformations similar to these are possible with proteins in various environments, it is concluded that the relative importance of side-chain and backbone contributions to wettability at protein–air interfaces is markedly dependent on polymer conformation. Investigations of poly(benzyl glutamate), in solvent-cast thin polymer films, showed that the hydrogen-bonding capacity of the common polyamide backbone could be masked by the bulky benzyl side-chain substituents without requiring configuration changes of the polymer chain. The influence of adsorbed water was apparently minor on these insoluble polypeptides under the conditions of this investigation but may be of greater importance with soluble biopolymers or in aqueous environments.

Despite the fact that proteins represent a class of polymers of vital interest in the biomedical field and also in certain industries, many of their surface properties remain to be determined. We believe that properly executed contact angle measurements with appropriate liquids at protein–gas interfaces could give helpful information about the polymer's outermost atomic constitution which would dictate interactions with its environment. However, it was not expected that contact angles (θ) measured on the various protein surfaces would be as readily obtained and interpreted as those for the numerous homopolymers and copolymers already investigated.[2] Since proteins consist of generally unknown sequences of about 20 different monomers arranged in a variety of configurations, the numerous reactions with, and interactions among, the many chain substituents are superimposed on the properties of the polyamide backbone common to all proteins.

This investigation focuses attention on a homopolymer of one of the amino acids similar to those found in proteins, and attempts to determine the influence which the chain configuration will have on the surface properties of membranes of this synthetic protein analogue. The current report builds upon earlier work on polyamides by Ellison and Zisman[3a] and on alter studies of polypeptides by Loeb and Baier[3b] and Baier and Zisman.[4] The polymer studied here is poly(γ-methyl L-glutamate), a well-known protein model[5] whose structural parameters are reasonably easy to change. We have taken advantage of reagent-induced alterations in the polypeptide conformation in this work, as we had with some success in our recent investigation.[3b]

Experimental Section

Materials. Poly(γ-methyl L-glutamate), PMG, was obtained in the form of a 4-mil thick sheet, cast from tetrahydrofuran, through the generosity of Dr. F. Reeder, Courtaulds, Ltd., Coventry, England. The material was free of surface-active contaminants;[6] after receipt, it was equilibrated at low pressure in an evacuation chamber to remove possible traces of the polymerization catalysts and stored in a refrigerator until use. Gel permeation chromatography of this polymer revealed an average molecular weight in the 300,000 range,[7] but a heterogeneity ratio of nearly 26 indicated a considerable fraction of lower molecular weight polymer.

Poly(γ-benzyl L-glutamate), PBG, was obtained in granular form from Mann Research Laboratories. Gel permeation chromatography of this material[7] yielded a weight-average molecular weight of about 300,000 and a heterogeneity ratio of about 5.

Chemical reagents used to induce or modify the polypeptide structural transformations were dichloroacetic acid, formic acid, and chloroform. Since initial experiments showed that redistilled solvents did not change the results obtained, reagent-grade chemicals were chosen for routine use. No systematic effects attributable to impurities in these reagents were found in either the contact angle data or the infrared spectra.

The surfaces of all films were washed gently with a soft, clean, camel's hair brush and a concentrated aqueous

(1) (a) NCR–NAS Postdoctoral Research Fellow at NRL. (b) To whom correspondence should be addressed.

(2) W. A. Zisman, Advances in Chemistry Series, No. 43, American Chemical Society, Washington, D. C., 1964, p 1.

(3) (a) A. H. Ellison and W. A. Zisman, *J. Phys. Chem.*, **58**, 503 (1954); (b) G. I. Loeb and R. E. Baier, *J. Colloid Interfac. Sci.*, **27**, 38 (1968).

(4) R. E. Baier and W. A. Zisman, submitted for publication.

(5) C. H. Bamford, A. Elliott, and W. E. Hanby, "Synthetic Polypeptides," Academic Press, New York, N. Y., 1956.

(6) F. Reeder, private communication.

(7) Waters Associates, Framingham, Mass.

TABLE I
CONTACT ANGLES ON POLY(γ-METHYL L-GLUTAMATE) IN VARIOUS CONFORMATIONS

Sessile drop of liquid (surface tension γ_{LV}, dyn/cm at 20°)	Average contact angle,[a] θ, deg: α sheet (a)	β sheet (b)	Mixed α/β sheet (c)	β sheet modified by chloroform (d)	β sheet after randomizing treatment (e)	α sheet on water (f)
Water (72.8)	57	74	62	67	58	58
Glycerol (63.4)	54	72	59	65	51	52
Formamide (58.2)	42	65	47	53	39	43
Thiodiglycol (54.0)	29	57	41	37	28	27
Methylene iodide (50.8)	42	53	40	45	43	42
Ethylene glycol (47.7)	30	47	30	29	25	33
sym-Tetrabromoethane (47.5)	35	44				
1-Bromonaphthalene (44.6)	25	33	14	32	17	24
o-Dibromobenzene (42.0)	18	30				
Tri-*o*-cresyl phosphate (40.9)	15	30				
1-Methylnaphthalene (38.7)	5	26				
Dicyclohexyl (33.0)	0	12				
n-Hexadecane (27.7)	0	0	0	0	0	0

[a] Reported values are averages of at least ten readings on at least two independently prepared films of each type.

solution of the detergent Tide,[3a,4] followed by copious rinsing with distilled water and equilibration at 50% relative humidity and 20° in a clean room, just before measurements.

Sessile drops used in the contact angle measurements included nonhydrogen-bonding and hydrogen-bonding (H-bonding) liquids covering a broad range of surface tensions and a variety of structural types. Sources of each liquid, its purity, and its surface tension (γ_{LV}) at 20° are detailed elsewhere,[8,9] but for convenience the liquids used and their surface tensions are listed in decreasing order of surface tension in the first two columns of Table I.

Methods. The PMG film, as received, was translucent with a smooth, shiny surface suitable for contact angle measurements and infrared surface spectroscopy directly after cleaning. The sample was completely soluble in both chloroform and dichloroacetic acid. The film surface could be modified without affecting the bulk significantly by placing a small droplet of chloroform or dichloroacetic acid on the film and then allowing the solvents to evaporate slowly from the surface in a large, covered, grease-free container.

Only a very small fraction of the PMG, as received, was soluble in formic acid; this was presumably the lower molecular weight fraction. The bulk of the material swelled in formic acid, maintaining its geometric form, to about 1.5 times its initial dimensions. Generally, two or more changes of soaking solvent sufficed to remove completely soluble components. Very thorough rinsing of the dried films involving multiple changes of rinse water or continuously flowing water was required to rid the structurally transformed films of residual formic acid; the retention of formic acid in the films was readily detected by contact angle measurements during which all of the hydrophilic liquids would spread rapidly over the surface when the formic acid had not been completely removed.

Upon drying the formic acid-soaked PMG films directly from the formic reagent, they returned to approximately their original dimensions; they were, however, more transparent, slightly thinner, and more brittle than before the formic acid treatment. Moreover, they were completely insoluble in chloroform while retaining their solubility in dichloroacetic acid. Three methods of drying were found suitable for forming modified PMG films which retained the smooth surfaces required for contact angle and infrared studies. Method A, the most often used, was that of gently stretching the film, before soaking, over a Teflon O ring and securing it in place with a close-fitting, slightly larger Teflon O ring; the film was not removed from this clamping jig at all throughout the soaking, drying, and rinsing steps. Such films generally became taut and hard, with smooth surfaces in their central regions; an additional advantage was that the PMG film portion clamped between the Teflon rings was not exposed to the transforming effects of the reagent, retaining all its initial properties and serving as an effective internal control. On this type of film, representative contact angles were measured on both the modified PMG central region and on the control (unmodified) annulus surrounding it. Using method B, the swollen, fragile film during drying could be pressed flat between acid-cleaned glass slides; the disadvantage of this method was the extremely long drying time required, because the only evaporation of solvent was from the film edges; the advantage was that large, flat, smooth films could be obtained. Method C was to allow the swollen film to be dried on a single acid-cleaned glass slide, or alternatively against the side of a clean beaker (say, the soaking beaker); this was the least satisfactory of the three methods because of the severe curling of film edges during drying. No differences were detected between the film surfaces dried at the air and glass interfaces.

To measure the wettability of films saturated with water, each film was clamped over a clean nylon disk (using a Teflon O ring) and distilled water was introduced between the film and nylon surface so that the water was held in place by capillarity for a long time. After a few hours had been allowed for absorption equilibration under these conditions, contact angles were measured on the upper film surface—some 0.004 in. away from the bulk water contacting the lower surface of each film.

Thin films of each polymer PMG and PBG were formed on polished platinum plates or sheets by the technique illustrated in ref 4. Films once cast on platinum sheets were further modified by soaking each, while still adhering to the platinum, in formic acid to induce configurational changes.

The slowly advancing contact angles of each of a series of pure liquids were determined on all specimen surfaces using the slow drop–buildup method[10] and an NRL goniometer telescope.[11] Each contact angle value recorded is

(8) H. W. Fox and W. A. Zisman, *J. Colloid Sci.*, 5, 514 (1950).
(9) F. Schulman and W. A. Zisman, *ibid.*, 7, 465 (1952).
(10) E. G. Shafrin and W. A. Zisman, *J. Colloid Sci.*, 7, 166 (1952).
(11) NRL Contact Angle Goniometer, commercially available from Ramé-Hart, Mountain Lakes, N. J.

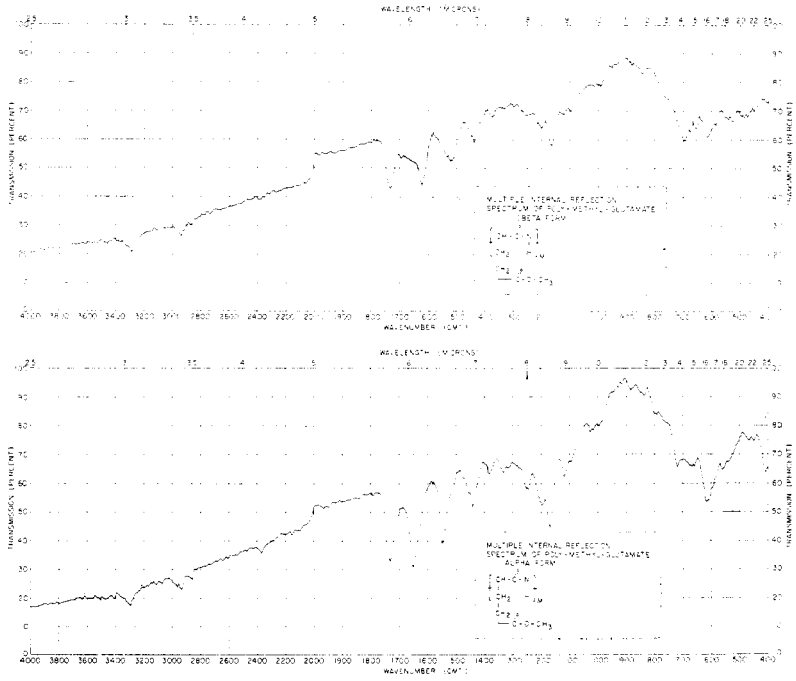

Figure 1. Internal reflection spectra of α and β forms of poly(γ-methyl L-glutamate) film.

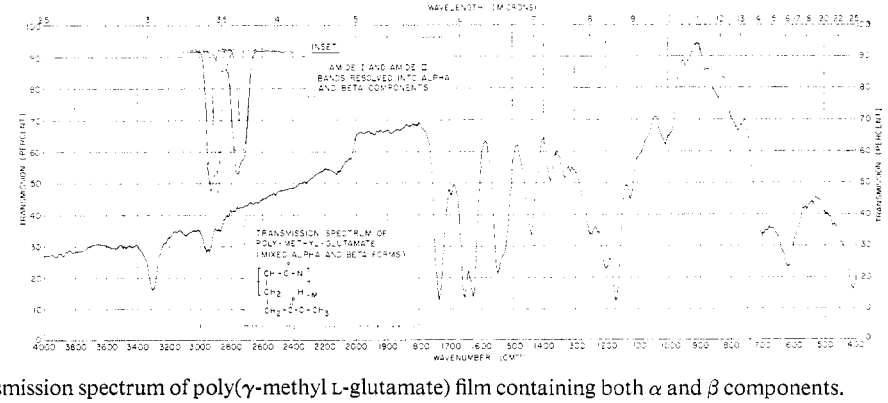

Figure 2. Transmission spectrum of poly(γ-methyl L-glutamate) film containing both α and β components.

that which occurred reproducibly within the first 10–20 sec after slowly advancing the sessile drop over a fresh surface region. Contact angles exhibited by water, glycerol, formamide, thiodiglycol, and ethylene glycol diminished very slowly after this time because of a very slow penetration of each liquid into the plastic solid. In addition, the thiodiglycol and the ethylene glycol each left a whitish opaque swollen region in all PMG films, regardless of structural modification, when they were "wicked" from the surface with filter paper. Furthermore, the latter two liquid occasionally showed a drop–buildup effect; that is, as a sessile drop was built up by the addition of more liquid, it did not immediately advance over a new surface area but only increased in volume (and contact angle) until mechanically disturbed. This phenomenon has previously been observed where sessile drops of certain liquids exhibited strong interactions with the polymer surface. For example, Ellison and Zisman[3a] had reported such drop–buildup with certain organic solvents on polystyrene and polyethylene terephthalate, and we have found it to be common with H-bonding liquids on several protein-aceous surfaces.[12] In the investigation being reported here, PMG films swollen in isolated spots with glycerol showed—in those same spots—methylene iodide contact angles typically 15° lower than those reported in Table I which characterized the nonswollen film areas.

Multiple attentuated internal reflection (MAIR) infrared spectra of the polymer film surfaces were obtained with the aid of a Wilks[13] Model No. 9 internal reflection accessory and KRS-5 internal reflection prisms (50 × 20 × 2 mm). Although Perkin-Elmer 21 and 457 and Beckman IR-5 and IR-12 infrared spectrophotometers were used to varying degrees throughout this investigation, the spectra reproduced in Figures 1 and 2 were traced from recordings generated on the last named instrument.

Results

Surface Classification by Internal Reflection Spectroscopy. (a) Bulk Films. The MAIR infrared spectra allowed the classification of the variously modified PMG films into three types; for convenient reference, these are designated here as α film, β film, and mixed α/β film. As illustrated in Figure 1, top and bottom traces, numerous absorption differences characterized the two major structural plastic forms of this polypeptide. The lower trace in Figure 1, which is characteristic of the polymolecular films designated as the

(12) R. A. Baier and W. A. Zisman, presented to the 143rd National Meeting of the American Chemical Society, Miami Beach, Fla., April 1967.

(13) Wilks Scientific Corp., South Norwalk, Conn.

α form, has major diagnostic peaks at 1654, 1550, and 625 cm^{-1} which are diminished or absent in the β-form traces. This spectrum for α-type material was obtained for the PMG, as received, after modification with chloroform, or after modification with dichloroacetic acid (DCA). There were some minor differences in the 1500–1700-cm^{-1} region, and in the 600–700-cm^{-1} region, after treating surfaces with DCA, usually accompanied by band broadening, which were not reliably reproduced from experiment to experiment in these studies. Since the shifts, if real, were minor and since the wettability of these DCA-modified films was quite similar to that for clearly α-type films, the DCA-treated PMG specimens are also fairly classified as α type for the purposes of the current analysis.

In contrast, the β modification had diagnostic bands at 1700 (weak, but always present), 1630, 1525, and 700 cm^{-1}. The upper trace in Figure 1, which illustrates a typical β-type spectrum, characterized only those films which had been swollen and dried from formic acid. It should be evident from an inspection of the shoulders on the bands in the 1500–1700-cm^{-1} region, and from the continued appearance of a markedly weaker band at 625 cm^{-1}, that some α-type polypeptide remained in these films. Since the depth of penetration of the infrared signal into the sample was of the order of 1 micron for the conditions used in these experiments,[14] and since the surface wettability for the β modification was completely different from that for the α type, it is reasoned that the remaining α-type material was present somewhat beneath the outermost molecular layers. On the other hand, soaking of these β sheets for long periods of time in chloroform prompted a return of the wetting characteristics of the film surfaces to that of the α sheets with only a small increase in the strength of the infrared absorptions correlated with the α type of structure. Solubilization of the surface of β sheets in DCA, and subsequent drying from that solvent, produced a complete return of the spectrum to that of the α type.

The absorption bands listed above are commonly taken as diagnostic for different backbone conformations of polypeptides and proteins[5,15] as will be discussed later. There were also some differences apparent between α and β films in the other regions of the spectrum, notably in the absorption intensities between 1100 and 1500 cm^{-1}, but these were not used in the present work to discriminate among samples modified with the various reagents.

Spectra showing various mixtures of α- and β-type components characterized PMG films only partially modified with formic acid, *i.e.*, usually produced by a briefer than normal swelling period or by not providing multiple changes of the formic reagent. Films with spectra showing both α- and β-type absorption bands were designated mixed α/β sheets. PMG films formed upon evaporation of the formic acid soaking solution, which had obviously solubilized some of the polypeptide from the starting sheet, had exclusively α-type spectra and wettability.

(14) N. J. Harrick, "Internal Reflection Spectroscopy," Interscience Publishers, New York, N. Y., 1967.
(15) T. Miyazawa, "Aspects of Protein Structure," Academic Press, New York, N. Y., 1963.

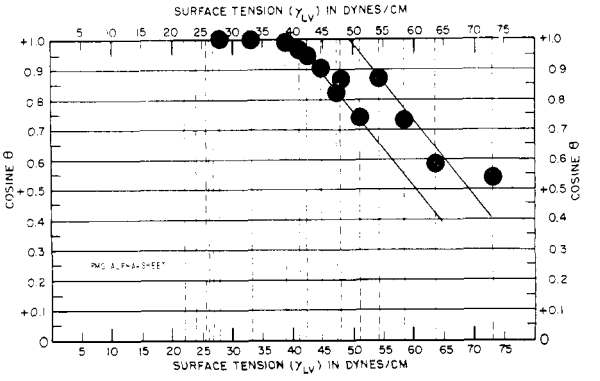

Figure 3. Plot of contact angle data for PMG film in the α conformation.

It was important to know if the MAIR spectra could be reliably used to indicate polypeptide conformations previously correlated with infrared transmission spectra. To establish that the peak positions were indeed the same with no major shifts or skewing in reflection and transmission modes, sample films were usually scanned in each mode and compared. Figure 2 shows the transmission spectrum for one such film, a mixed α/β sheet. It is apparent that all the diagnostic peaks are present, and in the same positions as obtained with the internal reflection accessories. The inset on this figure shows the amide I and II bands that is, the bands between 1500 and 1700 cm^{-1}, which were resolved (with the aid of a Du Pont Model 310 curve resolver) into their major components. This band analysis shows that about 40% of the absorption is due to peaks correlated with the α spectrum and about 43% of the absorption can be ascribed to the film fraction in the β configuration. Only 17% or so of the absorption occurs in an unassigned intermediate band around 1600 cm^{-1}, which does not include the β-related 1700-cm^{-1} peak mentioned earlier. Thus, only two major configurations seem to be present in the mixed α/β sheets; the absorption bands for these differing structures are reliably indicated in the internal reflection spectra and so may be used for structure assignment or classification on the basis of previous experience with transmission infrared spectroscopy.[5,15]

(b) MAIR Spectroscopy of Solvent-Cast PBG and PMG. In the case of the solvent-cast polypeptides, and especially PMG, variations in the solvents produced variations in the polymer configuration in the dried film.

PBG films cast from dichloroacetic acid produced spectra typical of the α-helix (or random-coil chain) configuration. PMG films cast from dichloroacetic acid, chloroform, and formic acid also gave this α-type spectral pattern—indicative of coiled chains intramolecularly hydrogen bonded. The formic acid cast films of PMG, in particular, gave sharp narrow absorption bands typical of highly α-helical polypeptides. On the other hand, PMG swollen in formic acid (but not soluble in this reagent) converted to the β-type spectrum.

Surface Classification by Wettability Criteria. (a) Bulk Films. The contact angle values characteristic of films of the α-modification are given in the column labeled (a) in Table I. These data are plotted as cos θ *vs.* γ_{LV} in Figure 3. It is clear from this graph that

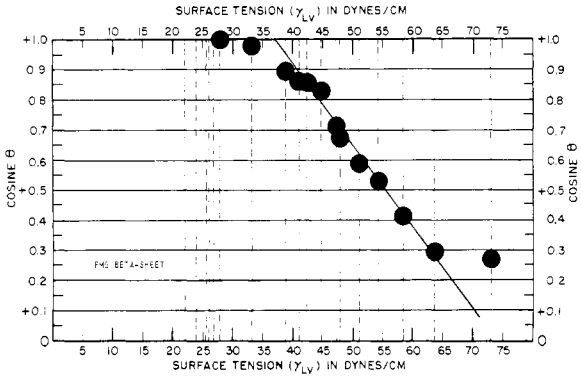

Figure 4. Plot of contact angle data for PMG film transformed to β conformation.

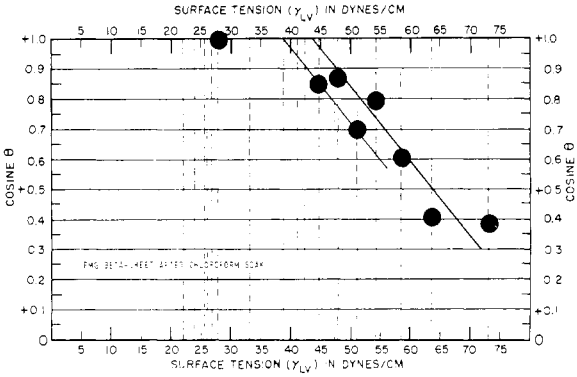

Figure 6. Plot of contact angle data for PMG film partially transformed from β to α form by chloroform.

a significant difference in the contact angles occurs between hydrogen-bonding and nonhydrogen-bonding liquids. The critical surface tension (γ_c), or the intercept at the cos θ = 1 axis, in the range of 40–50 dyn/cm, is not unusual for polyamides,[3b,4] and the presence of two distinct intercepts for the two different classes of wetting liquids is also a common feature for amide-based polymers. This type of wetting behavior characterized all films as received and after surface modification by chloroform.

The column labeled (b) in Table I lists the average contact angles for PMG films converted to the β form by swelling in formic acid and drying according to the procedure described. Plots of these data in Figure 4 reveal that the critical surface tension intercept dropped substantially out of the range common with polyamides and that the "dual wettability" feature associated with an accessible polyamide backbone was eliminated in the β modification.

Column (c) of Table I and Figure 5 include the contact angle data for mixed films of the α/β sheet form, created by partially treating α sheets with formic acid. It is apparent that some shift in wetting behavior toward that characteristic of β sheets had occurred, but that α-type wettability prevailed. Infrared reflection spectra showed that this high critical surface tension range and "dual wettability" prevailed even when there were very small fractions of α-class polymer in the specimen surface.

Column (d) of Table I and Figure 6 present the results for β sheets which had been soaked in chloroform for at least 48 hr. Chloroform is an excellent solvent for these films of PMG when in the α configuration, but the β sheets floated at about middepth in pure chloroform for indefinite periods without any visual change. In only one instance was a slight swelling noted. The contact angles and the cos θ *vs.* γ_{LV} data plot show that a partial return to α-type wettability occurred; the surface spectroscopy showed a concurrent, but still minor, increase in the absorption due to α-type polypeptide chains.

The data in Table I, column (e), are representative of the β sheets after they had been solubilized and dried from dichloroacetic acid which is a commonly used randomizing solvent for polypeptides. The generally lower contact angles, the higher critical surface tensions, and the large contact angle differences between H-bonding and non-H-bonding liquids all attest to the return of wettability characteristic of the α-type film. Indeed, the lower contact angles are what one might expect for a more open, randomized, network of polyamide chains even though no significant differences in infrared spectra were recorded for DCA treated and other α-type films. The cos θ *vs.* γ_{LV} graph for these films is given in Figure 7.

The final column in Table I, column (f), lists the contact angles obtained on the surfaces of α sheets which were equilibrated with their lower surfaces in contact with water. Comparison of these values with those for the "dry" α sheet given in column (a) shows that the immediate presence of bulk water did not influence the wettability of these films. Similarly, β

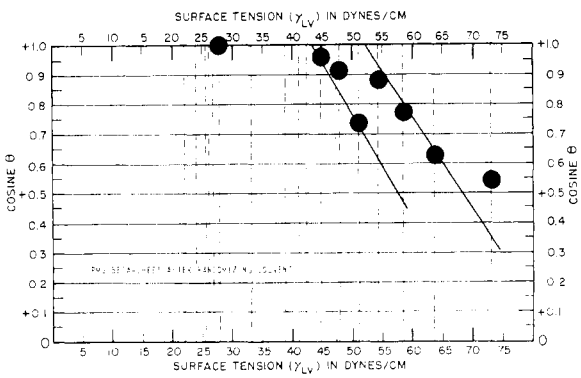

Figure 5. Plot of contact angle data for PMG film containing both α and β components.

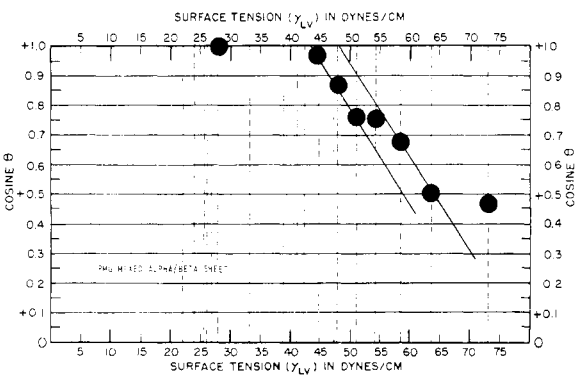

Figure 7. Plot of contact angle data for PMG film transformed from β to α form by dichloroacetic acid.

TABLE II
CONTACT ANGLES ON POLYPEPTIDE FILMS SPREAD FROM SOLUTION IN VARIOUS SOLVENTS ON PLATINUM PLATES

Wetting liquid (surface tension (γ_{LV}), dyn/cm at 20°)	Average contact angle,[a] θ, deg					
	Polyglycine (Nylon 2) (a)	PMG (a)	PBG (a)	PMG (c)	PMG (d)	PMG (b)
Water (72.8)	49	69	71	66	76	49
Glycerol (63.4)	38	59	63	65	69	47
Formamide (58.2)	30	48	63	52	62	34
Thiodiglycol (54.0)	23	39	44	40	46	29
Methylene iodide (50.8)	30	42	38	39	35	32
Ethylene glycol (47.7)	25	31	44	38	37	23
1-Bromonaphthalene (44.6)	10	25	25	26	24	13
Hexadecane (27.7)	0	0	0	0	0	0

[a] Reported values are averages of at least ten readings on at least two independently prepared films of each type: (a) cast from DCA; (b) cast from formic acid; (c) cast from chloroform; (d) swelled in formic acid.

sheets were unperturbed when in contact with water on their under surfaces—only some 3–4 mils away from the upper faces upon which contact angles were measured.

(b) Contact Angles on Polypeptides Cast from Dichloroacetic Acid (DCA). Polyglycine formed smooth translucent films which adhered tenaciously to the platinum sheets when spread from a solution in DCA. Experimental observations reported in ref 4 and contact angles are summarized in Table II for purposes of comparison.

PMG, which differs from polyglycine only in having a methylglutamate ester side chain substituted for one of the hydrogen atoms on the carbon separating amide links, formed smooth transparent films when cast from solution in DCA. Contact angle results for these films are also given in Table II.

PBG differs from the preceding PMG polymer only in that a benzyl ester side chain of glutamic acid is present rather than the methyl ester. The contact angle results obtained with films formed by drop-spreading PBG from dichloroacetic acid are also reported in Table II. These were also smooth, transparent films which adhered well to the platinum foils.

(c) Contact Angles on PMG Films Cast from Chloroform. Whereas the strong action of dichloroacetic acid is thought to induce the normal statistical random-coil type of configuration in polypeptide solutions, there is good evidence[3b] that PMG dissolved in chloroform possesses a high degree of the ordered α-helix chain structure. Contact angle results obtained with PMG films cast from chloroform are given in Table II.

(d) Contact Angles on PMG Films Swollen in Formic Acid. Highly-polymerized PMG is not soluble in formic acid; as noted earlier, it does swell in this reagent, however, and upon drying assumes an extended-chain configuration characterized as the β or extended structure. It was thus possible by formic acid swelling of PMG films cast from chloroform to obtain contact angle results for both extreme forms of polymer configuration (the as-cast α-helical form and the after-swelling β-structure form) from the same thin cast film.

These results are compared in columns 5 and 6 of Table II. PMG films on platinum sheets after being cast from chloroform were immersed halfway in 100% formic acid for 48 hr. The PMG film was thoroughly rinsed and allowed to air dry and was then cleaned with the standard detergent solution in water. The upper portion of the film remained unmodified and so could serve as an internal control for each experiment. The unmodified portion of each film retained its original transparent appearance and gave the same contact angles as recorded before soaking, whereas the portion modified by formic acid assumed a translucent appearance and gave the markedly different contact angles recorded in Table II.

(e) Contact Angles on PMG Cast from Formic Acid. Since a low molecular weight fraction was present in the PMG sample, and since this fraction showed an apparent ready solubility in formic acid, it was possible to drop-spread PMG films from formic acid directly. The formation of such films illustrates an important feature of the drop-spreading technique outlined earlier:[4] fractionation of the polymer by differential solubility occurred, particularly when poor solvents were used. Films of the formic acid-soluble fraction of the PMG were formed by allowing drops of the solvent to swell and dissolve partially a piece of the bulk PMG film placed in the center of a flamed platinum sheet. The formic acid which spontaneously spread to cover the metal sheet carried with it some of the dissolved polypeptide, and after being air-dried left PMG deposited as a very smooth outermost translucent film. Films identical in all respects could be formed by allowing the formic acid soaking bath from part (d) to evaporate to dryness on any clean flat surface. The last column of Table II summarizes the results obtained from contact angle measurements on these films.

(f) Comments. Some final comments on the contact angle values are now in order. In general, the spot-to-spot variability of contact angle measurements on a given surface was less than 2°; from sample-to-sample of each type, occasional deviations from the average as large as 5° were noted, but in every case the cos θ *vs.* γ_{LV} plots for α sheets showed dual lines and for β sheets a single line with a markedly lower γ_c intercept. Formamide occasionally penetrated very rapidly with no apparent reason on the β sheets, as judged by the relative stability of the other wetting liquids; some residual formic acid, or pockets left as the formic acid evaporated, would explain this observation. Also, in

the case of β sheets, hexadecane occasionally showed an initial contact angle of about 10° (rather than spreading rapidly, as usual) which decreased to 0° very slowly; this is also unexplained. An additional peculiarity was that in some cases, but not all, ethylene glycol exhibited greater than expected contact angles and thus plotted more in line with the non-H-bonding liquids than the H-bonding liquids.

Discussion

It is essential, first, to assess the reliability of the results and their freedom from artifacts. As the accumulated results show, α-type wettability—that is, a disparity in the wetting of the polymer surface by H-bonding and non-H-bonding liquids—could be approached from either direction in mixed films. That β-type films could be restored to exhibit α-type wetting, by treatment with either dichloroacetic acid or chloroform, is especially good evidence that the formic acid solvent (and swelling agent) did not remove from the films any special components (say, the low molecular weight fraction) with inherent α-type properties—leaving behind only irreversibly β-structured polypeptide. That the polypeptide films formed by drying the formic acid swelling solution itself were of the α type in both spectrum and wettability is convincing evidence that the formic acid did not carry a hydrophobic contaminant which became localized at the polymer surface to inevitably give β-type wetting and spectra. Indeed, the cyclic process of transforming α-type films to β type (by formic acid swelling) and then back to α type (using the α-helix-favoring solvent, chloroform, or the randomizing solvent, dichloroacetic acid) could be correlated routinely with both MAIR infrared spectroscopy and contact angle measurements. The β-type wettability always accompanied a β-type surface spectrum, for example, even when transmission spectroscopy showed considerable, and sometimes only, α-type material remained in the bulk film. Conversely, even a slight increase in α-type absorption in the surface spectra (for example, after a chloroform soak) correlated with a return of the surface wettability to α type, whereas the transmission spectrum through the bulk film remained apparently unchanged.

Thus, the results recorded appear to be substantially free from possible artifacts; the actual polymer modification at the specimen surface seems to be all-important.

The immediate environment of the polymer surface matrix determined the resultant configuration or mixture of configurations which obtained in our measurements. For instance, simple dilution of the formic acid soaking solution with water led to a dramatic precipitation of a frothy white mass of swollen polypeptide which, upon drying, showed a broad mixture of configurations by both transmission and surface infrared spectroscopy. If the bulk sheet was still present in the formic acid (*e.g.*, while still swelling) at the point of water dilution, its surface was similarly modified from the β type to a broad mixture of configurations (determined by MAIR infrared spectra) typically with α-type wetting behavior; often this surface layer could be peeled from the bulk film to reveal an homogeneous β-type surface below the immediate surface film. These observations support the earlier conclusion of Loeb and Baier[3b] that the solvent mixture existing just at the moment of surface spreading on water is the structure-determining condition for protein and polypeptide monolayers.

Both spectroscopically and surface chemically, the free (bulk) PMG films resemble those films cast from solution. Our earlier work[3b,4] with polyamides noted that a common feature in the contact angle data for polymers with accessible polyamide backbones was that the cos θ *vs.* γ_{LV} plots (including data for the H-bonding and non-H-bonding liquids) divided into separate, nearly parallel, straight lines intercepting at different critical surface tensions. Since the γ_c intercept for the H-bonding liquids was always substantially higher than that for the non-H-bonding liquids, and also higher than commonly obtained with polymers free from amide groups, it was concluded that this split in the data plot represented a H-bonding functionality at the polymer surface which increased the free surface energy per unit area.[3b,4] This diagnostic criterion seems applicable here.

An immediate clue to the existence of H-bonding functionality at the polymer surface may be obtained by observing the contact angles of only two liquids. These liquids are (a) thiodiglycol, which has a high liquid–vapor surface tension at 20° of 54.0 dyn/cm, a large molecular size, and H-bonding ability because of its hydroxyl substituents, and (b) methylene iodide, which has a high liquid–vapor surface tension of 50.8 dyn/cm at 20°, large molecular size, but no known capability for entering into H-bonding interactions. The large molecular size of each of these diagnostic liquids mediates against erratic results which might be obtained as the result of penetration and/or adlineation of the wetting molecules with side chains in the polymer surface. The relative closeness of their surface tensions indicates that in the absence of specific interactions, their contact angles should be relatively close together when the two liquids are used as sessile drops on the same polymer surface. Actually, the liquid of higher surface tension, thiodiglycol, should exhibit a somewhat greater contact angle on a plastic surface than should methylene iodide; this has been the common observation in contact angle measurements performed on non-H-bonding materials over the past two decades.[2]

In Table I, two heavy horizontal lines have been drawn to focus attention on the contact angle values actually obtained for thiodiglycol and methylene iodide. Inspection of these two adjacent rows of data rapidly indicates the nature of the polymer surface dealt with, and more carefully described, by the complete series of wetting liquids. For example, in column a for the α sheet, the thiodiglycol value is substantially lower than that for methylene iodide; this suggests a stronger than normal interaction for the H-bonding liquid which would correlate with accessibility to the polyamide backbone of PMG in this configuration. In column b, the opposite relationship is true for the β sheet; here the normal relationship typical of non-H-bonding plastics obtains. In addition, the generally larger contact angles for each liquid correlate with a lower critical surface tension intercept for PMG in the β sheet form. The lower γ_c indicates a relative absence

of high-energy amide links in the polymer surface. In column c of Table I, the contact angles for these two diagnostic liquids on a mixed α/β sheet are almost identical, thereby suggesting the presence of some H-bonding capacity in the mixed specimen's surface but not nearly as much as with the α-sheet material. Modification of the β sheets by soaking them in chloroform promotes a return of the surface configuration to that having accessible amide groups for H bonding (as illustrated by the values in column d, Table I). Randomizing the surface layers of PMG β sheets with dichloroacetic acid creates an even larger disparity in the wetting results for thiodiglycol and methylene iodide which suggests an even more open molecular network in the polymer surface (column e, Table I). The values in column f, like those in column a, show clearly the lack of influence on the wetting results when the 4 mil thick sheets were floating on liquid water.

Of course, it is more reliable to use a complete series of wetting liquids in order to determine more precisely the critical surface tension (γ_c) characteristic of the polymer in each of its configurations rather than to rely so heavily on the values of θ and γ_{LV} of the two liquids discussed above. The simplicity of comparing values for only two liquids, however, recommends this approach for other laboratories desiring information on the surface properties of biopolymers, yet not equipped with as elaborate surface chemical facilities as ours.

Now that the contact angle results have been shown to be good evidence of considerable differences in the polymer conformations, it is desirable to seek independent evidence for the various chain conformations which may be involved. It is also of interest to look for this evidence in the immediate outermost molecular layers of the specimens with as little interference from the bulk material as possible.

Some additional attention to the infrared surface spectra, which provide useful clues to the polymer backbone structure, is therefore worthwhile. The N–H stretching band, which occurs in these materials at about 3300 cm^{-1}, is relatively constant from film to film and so does not offer much utility in attempts at structure analysis. Since our previous investigations[3b,4] showed diagnostic wetting criteria defining surfaces with and without H-bonding functionality, and since these criteria were met, respectively, with the α-type and β-type films produced here, it seemed probable that certain polymer configurations allowed ready accessibility of the amides at the plastic surface while others might have masked the polyamide backbone completely. The following spectral differences suggest that the polyamide backbone is accessible in the coiled polypeptide configurations, such as in the α helix and the random tangle, but it is masked in the extended chain β structure when side chains are present. The requirement for side chains is essential, since it has been shown (see Table II) that polyglycine (a polypeptide without side chains, but with a predominantly extended chain configuration) exhibited the dual wetting feature for the two classes of liquids.

The coiled configurations have been correlated[5,15] with amide I, amide II, and amide V absorptions at 1655, 1550, and 620 cm^{-1}, respectively. These coiled configurations include the regular spirals stabilized by intramolecular hydrogen bonds and the randomly coiled molecular forms; the films classified earlier as of the α type fit these absorptions quite well. Extended-chain polypeptide and protein configurations have been correlated[5,15] with amide I absorption at 1625 cm^{-1}, amide II absorption at 1520 cm^{-1}, and diminution of the amide V band at 620 cm^{-1}. The most common extended chain structure is designated the β structure, which is an array of adlineated polypeptide chains hydrogen bonded to one another primarily through intermolecular associations; the films classified earlier as β type fit the β structure spectra well. The two different configurations produced in the same specimen surface could also be distinguished readily by attention to these few specific bands. Unfortunately, no clear differentiation has yet been made among coiled configurations, and particularly between regular spirals, such as the α helix and random tangles. Studies of oriented specimens using polarized infrared techniques may eventually allow such differentiation. For present purposes, we have only two rather clear and unambiguous structural assignments which can be made using the spectral criteria.

The α-type spectrum is that which characterizes both the coiled configuration and the random tangle configuration, and this assignment has been made predominantly based upon observation of the amide absorption bands as discussed above. The most important coiled form included in this category is the intramolecularly H-bonded α helix defined by Pauling and Corey on the basis of X-ray evidence;[16] this explains our use of the term "α type" for these presumptively α-helical films throughout this report. Spectra characteristic of extended and intermolecularly H-bonded polyamide chains have similarly been called "β type" because the predominant chain configuration included in this class is believed to be the β structure, also defined by Pauling and Corey from their X-ray studies.[17] Our experimental work has shown, however, that it is extremely difficult to produce completely uniform samples in any one configuration. We have learned, on the other hand, that the relative amounts of given configurations can be so altered as to have one or the other overwhelmingly determine the surface properties of the polymer.

Corey–Pauling–Koltun molecular models[18] of the many possible configurations for PMG have been constructed and analyzed to ascertain which influences of the side chains might be responsible for the actual differences in wetting obtained.

Similarly scaled models of each of the wetting liquids employed were also constructed and attempts made to understand the different apparent accessibilities of these liquids to the polyamide backbone common to all configurations.

Figures 8 and 9 represent artist's sketches of these molecular models in both plan and perspective views. The top drawing in Figure 8 is a plan view of the α-

(16) L. Pauling, R. B. Corey, and H. R. Branson, *Proc. Nat. Acad. Sci. U. S.*, **37**, 205 (1951).
(17) L. Pauling and R. B. Corey, *ibid.*, **37**, 251 729 (1951).
(18) W. L. Koltun, *Biopolymers*, **3**, 665 (1965).

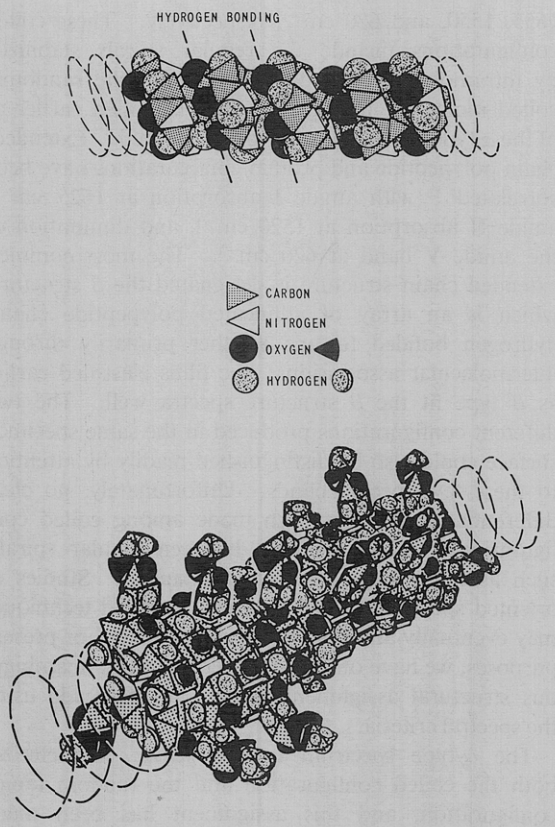

Figure 8. Sketches of molecular models of poly(γ-methyl L-glutamate) in α-helical conformation.

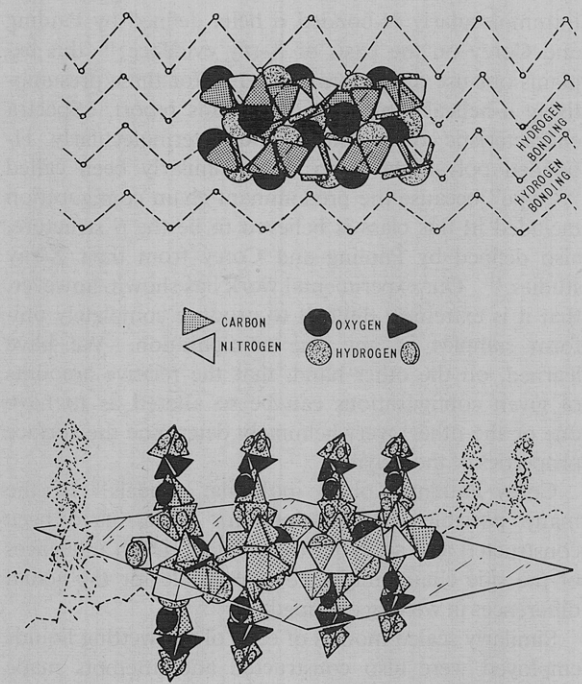

Figure 9. Sketches of molecular models of poly(γ-methyl L-glutamate) in the β structure conformation.

helix backbone only, with dashed lines suggesting the extension of the spiral in both directions and indicating the planes of H-bonding responsible for the helix's stability. The lower sketch in Figure 8 was drawn from this same molecular models after adding about two-thirds of the methyl glutamate side chains. It should be apparent from this drawing that the side chains in this coiled configuration do not offer much shielding of the polyamide backbone and its many potential sites for H-bonding interactions with wetting liquids. All of the pseudorandom configurations of this model which could be produced by uncoiling it and twisting it around gave equal, or substantially greater, accessibility of the amide–polymer links. Thus, one can understand the wetting results for α-helical and randomized PMG specimens which showed that there was ready accessibility of H-bonding liquids to the polyamide backbone according to the criteria established earlier.[4]

The top drawing in Figure 9 shows the polypeptide backbone (only) for the β structure, with the dashed lines very roughly suggesting the extent of the polymer chains in both directions and the locations of the interchain H-bonds responsible for this structure's stability. The lower sketch is a perspective view of this model with some of the methyl glutamate side chains added. The plane of the H-bonded pleated sheet is also indicated. It is to be noted that this configuration constrains the ester side chains to directions essentially perpendicular to the polyamide sheet. With all the methyl ester side chains in place, they form a relatively close-packed array which effectively shields the polyamide backbone from interaction with the wetting liquids. Although it is possible to distort the side chain packing in one area of the surface to form pores large enough for the entry of the smaller wetting molecules, this distortion causes even tighter packing of the methyl ester side chains in the adjacent areas of the polymer surface. Thus, the net effect of such distortion is not great enough to provide sufficient accessibility of H-bonding wetting liquids to the polyamide backbone to show up in the contact angle results.

This simple model analysis provides a plausible explanation for the differing wetting results obtained on α-helical and β-structured PMG and illustrates the potentially large influence with specific chain configurations may have on the surface interactions of proteins and other biopolymers. In the case at hand, the α-helical and random tangle films of PMG (a protein analog) should show wetting features similar to those obtained with the nylon-type polyamides and with polyglycine;[4] indeed, they do. Very little influence is apparent from the methyl ester side chains, Conversely, the model analysis reveals that the β structure for PMG predicts a wettability not significantly different from that obtained with other predominantly methyl ester-populated polymer surfaces; the excellent agreement between the contact angle results obtained here for β structure PMG and those earlier published for poly(methyl methacrylate),[19] which has no amide links at all in its structure, sustains this prediction. No evidence has been obtained that the γ-methyl ester of these polymers enters into H-bonding interactions with wetting liquids.

A problem area not adequately studied by the experiments reported here is that of the influence of adsorbed water on the surface properties of polymers. With

(19) N. L. Jarvis, R. B. Fox, and W. A. Zisman, Advances in Chemistry Series, No. 43, American Chemical Society, Washington, D. C., 1964, p 317.

the PMG films used here (about 4 mils thick) mounted taut and flat in contact with bulk water, the contact angle results did not differ significantly from those obtained when the same films were equilibrated at 50% relative humidity. These results may be contrasted with the earlier results with a water-soluble amide-containing polymer, poly(acrylamide).[19] The water solubility of this latter material suggests that it may have a greater affinity for water even in its solid form, and the observation that the organic liquids having lower surface tensions exhibited nonzero contact angles on poly(acrylamide) surfaces[19] is consistent with the presence of an adsorbed film of water.[20] The fact that the anomalous nonzero contact angles for organic liquids of low surface tension were dependent upon the relative humidity with which the polymer was equilibrated[19] is further support for the suggestion that surface adsorbed water is also influential in the wetting behavior of biomaterials. This additional factor must be considered along with configuration changes in the studies of natural materials. In subsequent wetting and spectroscopic studies[21] of the collagen-to-gelatin transition, we have found the influence of adsorbed water essentially superposed on the data typical of this ordered (collagen) protein and its disordered product (gelatin).

An additional problem area is that of differentiating the random tangle from the α-helix. Dichloroacetic acid is considered to be a general randomizing solvent for polypeptides, and should have produced a random tangle-type configuration in the dried films. However, the infrared spectra for random tangle polypeptide structures and for α-helical structures are nearly the same, and in the present investigation these two coiled types of configuration were not differentiated by MAIR spectroscopy. One would expect ready accessibility of the liquid to the polyamide backbone as the polypeptide was randomized, and such was the observation by contact angle methods. The possibility remains that in the course of being dried the polypeptides convert from their randomized solution structure to the more regular helical configuration. Whichever the case, it is interesting to note that a simple change in the nature of the side chain--replacing the methyl ester of glutamic acid with the more bulky benzyl ester—blocked the access of H-bonding liquids to the polyamide backbone chain. Since the MAIR spectra PBG films cast from DCA were practically identical with those for PMG cast from DCA, and yet the wettability results differed, it can be concluded that the bulky benzyl substituents masked the amides from the film surface by steric hindrance an not by a configurational change.

(20) E. G. Shafrin and W. A. Zisman, *J. Amer. Ceram. Soc.*, **50**, 478 (1967).
(21) R. E. Baier and W. A. Zisman, in preparation.

Conclusions

Samples of the synthetic polypeptide, poly(γ-methyl L-glutamate), were prepared in α-helical, extended-chain β structure, and random-tangle forms using common reagents. Cyclic transformations among these polymer conformations were also carried out. Structural assignments were made on the basis of multiple attenuated internal reflection infrared spectra of the modified surfaces, as compared with and contrasted to bulk transmission infrared spectra of the same films. It was shown that the helical and random configurations for this model protein had wettability characteristics similar to those of the nylons, indicative of accessible amide groups near the polymer surface according to previously hypothesized diagnostic contact angle criteria. The extended-chain β configuration showed wettability results characteristic of only the methyl-ester side chains, indicating little or no accessible amide links at the polymer surface. The side chains acted as an effective shield for the polyamide backbone.

Inspection of scaled molecular models of each of the configurations produced, with special attention to possible masking effects of the glutamate side chains, confirmed the analysis from the contact angle measurements.

The helical and random configurations both showed differences in wetting by H-bonding and non-H-bonding liquids, producing a range of critical surface tensions from about 40 to 50 dyn/cm. The β structure did not exhibit this preferential wetting by H-bonding liquids and had a single critical surface tension of about 40 dyn/cm or less.

Strong similarities in these results to earlier findings with some polyamides of the nylon class, and with results for polyglycine, support our earlier hypothesis that contact angle data may be used to diagnose for accessible H-bonding sites in a polymer surface.

Therefore, two major conclusions emerge from these studies of PMG and PBG: (1) the backbone configuration of a polypeptide, protein, or other biopolymer, can strongly influence that polymer's surface interactions, and (2) diagnostic contact angle criteria for the recognition of H-bonding functionality in a polymer specimen's surface are generally valid.

Remaining unresolved is the question of complications which may enter with water-soluble materials or with water-insoluble materials in aqueous environments.

Acknowledgments. Helpful suggestions were given by our associate, Dr. G. I. Loeb, and the cooperation of Miss Dorothy S. Cain of the Physical Chemistry Branch in obtaining the infrared spectra was particularly valuable. This work was carried out during the tenure of R. E. Baier as NAS–NRC Postdoctoral Research Associate, 1966–1968.