The pH-Induced Helix–Coil Transition of Poly-L-lysine and Poly-L-glutamic Acid and the 238-mμ Dichroic Band

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ABSTRACT: The pH-induced conformational changes of poly-L-lysine and of poly-L-glutamic acid have been investigated by CD spectral measurements in the wavelength region 200–250 mμ. The dichroic spectra of the nonionized polymers are typical of α-helically organized polypeptide structures characterized by a triply inflected spectrum (negative minima at 221–222 and 208–209 mμ and a positive maximum at 190–191 mμ). In the ionized form both polymers exhibit doubly inflected CD curves (small positive maximum at 218 mμ and a large minimum at 197–198 mμ). The dichroic changes at the 221-mμ extremum as a function of pH result in symmetrical S-shaped titration curves and the dichroic spectral titrations for both polymers exhibit a single point of crossing, an isodichroic point, at 204 mμ with an ellipticity of −20,400 ± 1400 deg cm^2/dmol. The linear combination of dichroic contributions from the limiting curves in proportions corresponding to the α-helical and uncoiled contents [estimated by the recently developed isodichroic method[17]] generates all the observed intermediate curves, including the usually reported triply inflected dichroic spectrum near neutral pH's. It is suggested that the pH-induced conformational change of the two electrolytes is a single-step process involving two conformationally distinguishable forms, *i.e.*, the α-helical and the uncoiled; the latter is characterized by a doubly inflected CD spectrum (small maximum at 218 mμ and large skewed minimum at 198 mμ). The 238-mμ minimum in the CD curves of polymers near neutral pH's is not due to any discrete electronic transition, *i.e.*, has no molecular origin, but is rather an artifact because of the summation of a small amount of α-helical structural contribution (2–5%) to a predominantly disordered form spectrum.

The elucidation of conformation of proteins and polypeptides has resulted in a significant concentration of effort toward the characterization of the optical activity spectra [optical rotatory dispersion (ORD) and circular dichroism (CD)] corresponding to various possible and/or occurring conformations of the polypeptide chains.[1–13] As a result, the optical spectra of solutions of synthetic polypeptides in various conformations have been examined, and the characterizing parameters (such as the positions of the optical bands, their rotatory strengths, etc.) and the general shape of the optical spectra have been identified.

The α-helical conformation in solutions is characterized by a positive dichroic maximum at about 190 mμ and well-defined negative extrema at 208–209 and 220–222 mμ,[2–6] whereas the pleated sheet or β conformation has a simple doubly inflected CD spectrum with a positive extremum at 195 mμ and a negative minimum at about 217 mμ.[7–10] The optical characterization of the so-called random form (or disordered conformation) is, however, still an unsettled issue. Dichroic measurements of poly-L-glutamic acid (PG) as well as of poly-L-lysine (PL) in solutions at neutral pH, in which the ionized polymer is considered to be in a random conformation, first lead to the characterization of its dichroic spectrum as being a doubly inflected spectrum with a large minimum at about 197 mμ and a weak positive maximum at about 217 mμ.[3,5,6,11] To this a third inflection, centered at about 238 mμ representing a rather weak band, was later added.[3,6,12,13] Recently, however, it has been suggested that the triply inflected dichroic spectrum of both polymers at about neutral pH is that of an intermediate structural state of the polypeptide chain, and at extreme pH's (low for PL and high for PG) the triple-inflected spectrum reduces to a doubly inflected dichroic spectrum,[14] which possibly represents an ordered structure similar to that exhibited by polyproline II.[6,15] These results, in accordance with those reported earlier,[3,12,13] do, however, characterize the dichroic spectrum of the polymer close to neutral pH, as a triply inflected spectrum with a positive peak at about 218 mμ, a large negative minimum at about 197 mμ, and a rather small negative minimum at 238 mμ. The presence of the negative minimum in the dichroic curves of the ionized polymer is not only unexpected,[3] but is also unexplainable by any of the existing theories attempting to describe the origin of optical activity in model polymers. In this com-

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TABLE I

CD PARAMETERS FOR α-HELICAL AND RANDOM FORMS OF POLY-L-LYSINE AND POLY-L-GLUTAMIC ACID

Band position, mμ (α-Helix)	Ellipticity, deg cm²/dmol × 10⁻³ (α-Helix)	Band position, mμ (Random)	Ellipticity, deg cm²/dmol × 10⁻³ (Random)	Position, mμ (ellipticity at isodichroic point, θ)
		Poly-L-lysine[a]		
221.5	−33.6	218	+3.8	
208	−32.0		−36.0	204 (−20,400)
191	+72.0	197		
Intercept at 201 mμ		Intercept at 211 mμ		
		Poly-L-glutamic Acid[b]		
221	−43.0	217	+6.3	
				204 (−21,000)
209.5	−38.0			
191	+89.0	198	−36.8	
Intercept at 202 mμ		Intercept at 210.5 mμ		

[a] α-Helical at pH 11.7 in water; uncoiled form at pH 4.5–7.5 in water. [b] α-Helical at pH 4.5 in water; uncoiled at pH 10.45 in water.

munication, we wish to note that, first, the pH-induced conformational transition of the two synthetic polymers, PL and PG, is a single-step process; second, the dichroic curve of the polyelectrolyte in the ionized form is a doubly inflected dichroic spectrum, with a maximum at 218 mμ and a large minimum at 197 mμ and not a triply inflected spectrum; and the so-called 238-mμ minimum (the third inflection of the spectrum) is not due to a specific optically active transition of a chromophore, but simply arises from the summation of small dichroic contributions corresponding to α-helical conformation in a predominantly random polypeptide chain.

Experimental Section

Poly-L-lysine (PL) hydrochloride (Mann Research Laboratory, Lot 53141; average molecular weight, 70,000) and poly-L-glutamic acid (PG) sodium salt (Sigma Chemical Co., Lot 1258-0360; average molecular weight, 68,000) employed in these studies were used without any further purification. The pH's of the polymer solutions were adjusted by dialysis against conductivity water, maintained at appropriate pH using a Radiometer pH stat; the final pH was measured on a Leeds–Northrop pH meter equipped with expanded scale. The concentrations of the solutions were determined spectroscopically using residue molar extinction coefficients reported by Holzwarth and Doty.[5] The circular dichroism measurements were made on the modified JASCO-ORD/CD/UV-5 spectropolarimeter.[16]

Results and Discussion

The dichroic curves of PG at different pH's between the limits 4.50 (representing the protonated form) and 10.45 (representing the completely ionized form) in the wavelength region 250–200 mμ in water are shown in Figure 1 and at the top of the figure are shown the expanded dichroic curves in the vicinity of neutral pH. Similar results for PL within pH limits of 11.7–4.5 are shown in Figure 2. In the insets in the two figures are plotted ellipticities at 221 mμ as a function of pH. In Table I are listed the molecular parameters characterizing the CD spectra at extreme pH's. The effect of increasing KCl concentrations on the CD spectrum of ionized PG (pH 8.4) in the region of the 218- and the 238-mμ inflections is shown in Figure 3.

Both polymers, PG and PL, in both the uncharged (curves A in Figures 1 and 2) and the charged (curve N, Figure 1; curve K, Figure 2) forms exhibit dichroic curves very similar to one another. Both polymers in the two forms exhibit dichroic curves with almost identical positions of the extrema and the crossover points,

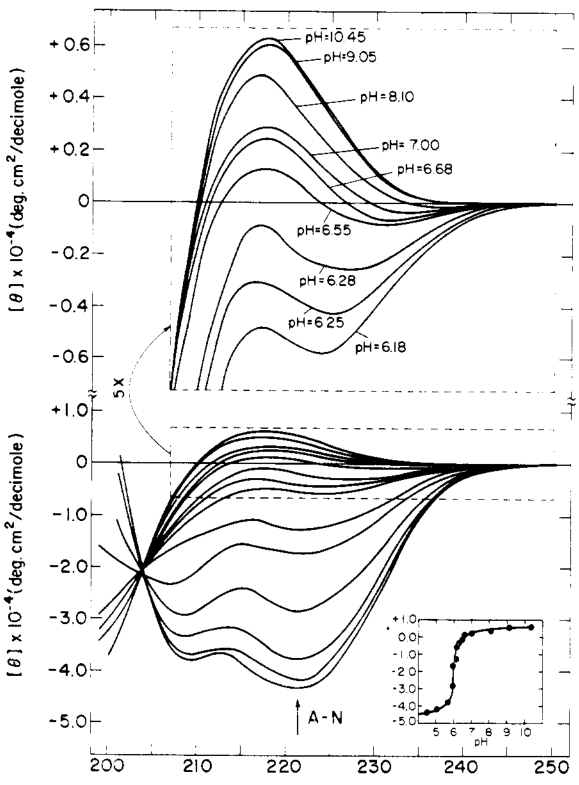

Figure 1. Effect of pH on the CD spectrum of poly-L-glutamic acid in water: concentration, 4.87 mg/10 ml; path length, 1–0.05 cm; temperature, 22°; A, pH 4.50; B, 5.02; C, 5.70; D, 5.90; E, 5.95; F, 6.10; G, 6.18; H, 6.25; I, 6.28; J, 6.55; K, 6.68; L, 7.0; M, 8.10; and N, 9.5–10.45; inset, change of ellipticity at 222 mμ as a function of pH.

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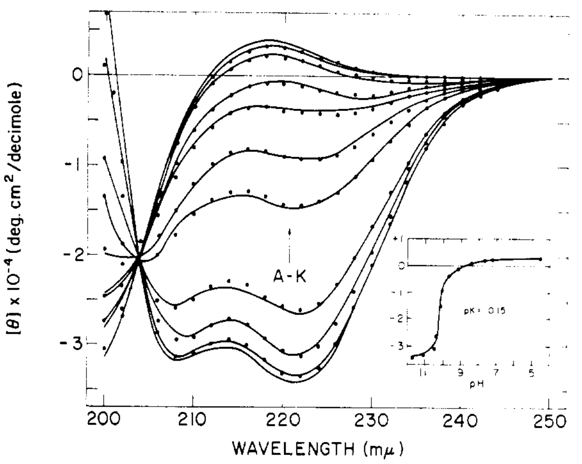

Figure 2. Effect of pH on the CD spectrum of poly-L-lysine in water: concentration, 4.36 mg/10 ml; path length, 1–0.05 cm; temperature, 22°; A, pH 11.70; B, 11.10; C, 10.45; D, 10.32; E, 10.15; F, 10.04; G, 9.64; H, 9.10; I, 8.35; J, 7.65; K, 7.0 and 4.5; ———, observed CD spectrum; ●, calculated dichroic curves by linear combination of appropriate proportions (refer to Table II) of contributions of dichroic curves at extreme pH (curves A and K); inset, change of ellipticity at 221 mμ as a function of pH.

as well as the positions and the magnitudes of the isodichroic points (Table I). These similarities are indeed consistent with the view that the two polymers exhibit similar conformational organizations, a right-handed α-helix in the uncharged form and possibly an extended form when charged.[1–6] This implies that similar electronic interactions among the peptide chromophores are responsible for the optical activity spectra of the two polymers. A comparison of the ellipticities at the extrema, however, indicates that PG exhibits larger ellipticities at the various extrema (Table I). Since the magnitude of ellipticity at an extremum is a reflection of the rotatory strength of the transition (a parameter directly related to the conformation of the molecule), the differences between PG and PL may be taken to indicate small, but definite, differences in the structural organization of the polypeptide chain in the two polymers. The fact that the two polymers reflect cases in which similarities in the shapes of the curves suggest similarities in polypeptide organizational structures as well as in the type of chromophoric interactions make it appear likely that the differences in conformation between the ionized forms, and similarly, between the structural organizations of the nonionized forms, are simply of relative nature. PG possibly forms a more compact helix when compared to PL, and similarly, a more extended form in the deprotonated form. This is supported by the observation that a given salt concentration causes larger changes in the ellipticities at the extremum of PG when compared to PL, and this is especially true in the case of the charged polymers. The relatively lesser stability of the PL α helix is also suggested from the observation that simply heating to 50° results in conformational transformation to β structure,[6–8] whereas there exists no such transformation in the case of PG.

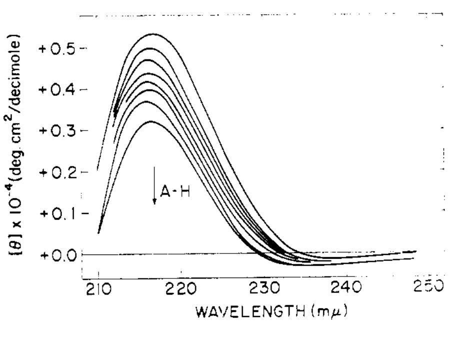

Figure 3. Effect of salt concentration on CD spectra of poly-L-glutamic acid in water at pH 8.45: concentration, 4.16 mg/10 ml; path length, 0.5 cm; temperature, 22°; A, no salt; B, 0.024; C, 0.101; D, 0.175; E, 0.316; F, 0.448; G, 0.627; and H, 0.937 mol/l.

Lowering the pH in the case of PG, and similarly, raising the pH of solutions containing PL, alter the doubly inflected dichroic curves to the typical α-helical dichroic curve with the formation of the commonly observed triply inflected spectrum[3,6,13,14] in the early stages of the titration (Figures 1 and 2). Almost comparable alterations of the CD curves have also been observed upon addition of organic solvents such as dioxane, ethylene glycol, chloroethanol, and methanol.[14] The changes in the dichroic curves upon addition of salts (Figure 3) also seem to indicate similarities to the changes observed in the early stages of the titration. The triply inflected CD spectrum generated during the early stages of the pH titration or upon addition of salts or upon addition of organic solvents to a salt-free solution is the spectrum which has been commonly suggested to represent the disordered form of the polymer,[3,6,12] and has recently been attributed to an intermediate structural state of the system.[14] Analyses of the profiles of the dichroic curves at various pH's (Figures 1 and 2), however, seem to suggest that, concurrent with the continuous increase of the ionization of the polymers, the 222-mμ α-helical minimum (representing predominantly n–π* amide contributions in α-helical array) undergoes a change reflecting its transformation to the inflection at 218 mμ. Similarly, the extremum at 209 mμ (Figures 1 and 2) as well as the 190-mμ positive extremum (Figure 4, transformation not shown) (representing contributions from the π–π* amide transitions) converge to generate the 197-mμ minimum. Almost identical (but in re-

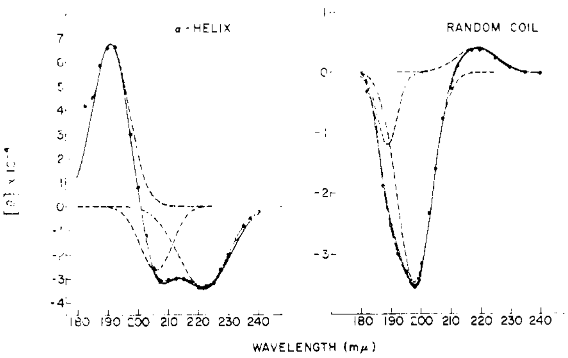

Figure 4. The observed CD spectrum, the Gaussian function and the generated spectrum of poly-L-lysine in water at extreme pH's: left, pH 11.7, α-helical; right, pH 7.0–4.5, random coil; —·—·—, observed spectrum; ———, Gaussian-generated CD spectrum; - - - - - -, Gaussian functions.

verse order) dichroic changes for PG have also been reported upon increasing concentrations of methanol in a solution at neutral pH.[14] The pH-induced conformational change, as well as that seen upon addition of methanol to neutral solutions, therefore, represents a continuous change of one conformational form of the polymer to another in which the two conformations are characterized by the dichroic curves at the limiting pH's in the two cases. The almost symmetrical S-shaped titration curves for the ellipticities at 221 mμ (insets, Figures 1 and 2) provide further support to the idea that the conformational transformation process is a simple single-step process involving only two conformationally distinguishable forms. This is further substantiated by the observation that, irrespective of the pH of the solution, or the conformational composition of the polymer or the nature of the model, all the dichroic curves (each representing an intermediate state) exhibit isodichroic points at the same wavelength, 204 mμ, which further maintains constancy of ellipticity in the two cases. The conclusion is therefore evident that the pH-induced conformational alteration process of the two models involves only two distinct dichroic entities, one characterized by the triply inflected dichroic spectrum, minima at 221–222 and 209–210 mμ, and a maximum at about 190–191 mμ, the α-helical conformation; and the other, by the doubly inflected dichroic curve, small maximum at 218 mμ and a large minimum at 197–198 mμ. The intermediate dichroic curves between the two extremes therefore simply reflect either gradually transformed forms from one conformation to another (if a cooperative mechanism is involved) or simply a composite of proportional dichroic contributions of the forms at the extreme pH's. This further suggests that the triply inflected CD spectrum at about neutral pH does not characterize a distinct conformational form, but possibly represents an intermediate containing some proportions of conformations characterized by the dichroic curves at extreme pH's. In this event, the so-called 238-mμ minimum would not be due to a specific optically active transition, but rather to the overlapping of dichroic contributions from small α-helical structures in predominantly disordered polymers.

A clear delineation of the above conclusion can be best established by comparison of the observed intermediate dichroic curves with those calculated by linear combination of the dichroic contributions in appropriate proportions from the dichroic curves at the extreme pH's. The conformational fractional compositions of PL in solutions at various pH's were estimated by using the recently developed isodichroic method,[17] and results are listed in Table II. The dichroic curves corresponding to various conformational compositions were constructed by first fitting the observed reference conformational curves (curves A and K, Figure 1) to Gaussian functions (Figure 4) and then summing the contributions corresponding to the fraction of each of the two conformations for the system at a given pH (Table II). The calculated results are superimposed on the experimental data in Figure 2, and the constructed curves for the predominantly disordered model containing incremental amounts of α-helical contributions (2–12% in increments of 2) are shown in Figure 5. The agreement between the observed and the calculated results (Figure 2) over the entire pH range, including the position of the magnitude of the isodichroic point, therefore confirms the conclusion that the pH-induced conformational change of these polyelectrolytes is indeed a single-step process and involves only two conformationally distinct species. The presence of any structurally distinct intermediate form characterized by a triply inflected dichroic spectrum (inflection points at 238, 218, and 197 mμ) therefore seems rather unlikely. In addition, the observation that CD curves similar to the observed triply inflected dichroic curves near neutral pH's (Figures 1 and 2) can be generated simply by linear combination of appropriate proportions of α-helical contributions (2–5%) with the contributions of the ionized polymer at extreme pH (Figures 2 and 5) clearly reflects the fortuitous nature of the 238-mμ minimum. In view of this, it seems reasonable to suggest that studies reporting the presence of a minimum at about 238 mμ in the CD curves of ionized polymers[3,5,11,13,14] are those of systems containing small proportions of α-helical conformation. Such

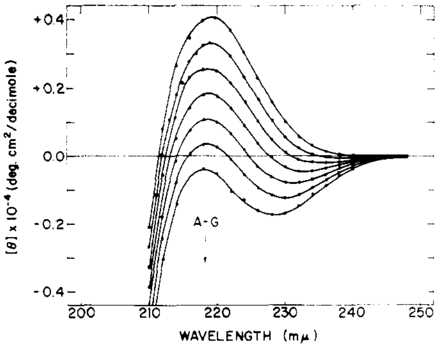

Figure 5. Computed CD spectra of poly-L-lysine containing increasing proportions of α-helical contributions: A, 100% random; B, 2% helix + 98% random; C, 4% helix + 96% random; D, 6% helix + 94% random; E, 8% helix + 92% random; F, 10% helix + 90% random coil; and G, 12% helix + 88% random coil.

TABLE II
CONFORMATIONAL COMPOSITION[a] (PER CENT) OF POLY-L-LYSINE AT DIFFERENT pH'S

pH	α-Helix	Random coil
11.70	100.0	
11.10	99.0	1.0
10.45	92.1	7.9
10.32	78.2	21.8
10.15	48.2	51.8
10.04	34.2	65.8
9.64	21.0	79.0
9.10	14.0	86.0
8.35	5.4	94.6
7.65	2.3	97.7
7.0–4.5		100.0

[a] Estimated by the isodichroic procedure. Expression used $f_\alpha = ([\theta]_{208}{}^{\mathrm{obsd}} - [\theta]_{208}{}^{\mathrm{max}})/([\theta]_{208}{}^{\alpha} - [\theta]_{208}{}^{\mathrm{max}})$, where $[\theta]_{208}{}^{\mathrm{max}}$ is the ellipticity of the isodichroic point, −6100 ± 300 deg cm^2/dmol, and $[\theta]_{208}{}^{\alpha}$ is the ellipticity of the α-helical model at 208 mμ, −32,000 ± 1500 deg cm^2/dmol (see ref 17).

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a situation could arise simply from incomplete unfolding of the polypeptide chain or from the presence of salts which have been shown to affect the magnitude of the dichroic curves, presumably by a shielding effect.

The two-banded spectrum of PG and PL in the ionized form has a strong resemblance to the dichroic curves exhibited by polyproline II,[3,6,15] which has been shown to exist in a threefold helix. This raises questions regarding the validity of assignment of the random form to the ionized polymer. In view of the facts that the electrostatic interactions of the side chains tend to favor extended chain configuration of the polymer, and that both extensive hydrodynamic[1-3] and recent nmr studies[18] suggest that the charged polymer is indeed in extended configuration, and since the positions and the rotatory strengths of the optical bands (parameters specifically characterizing the conformation of a given system) of the charged polymers are significantly different from those exhibited by the threefold helical model, polyproline II,[3,6,15] and because of the absence of any other additional evidence suggesting to the contrary, we hold the opinion that the doubly inflected dichroic spectrum of the ionized polymers in a salt-free medium is that of the disordered form. It is still to be seen whether or not the disordered conformation of the ionized polymer indeed represents a true random form. A note of caution is further called for when speaking of "random" or "disordered" or "uncoiled" forms of polypeptides, since the conformation, and thus the optical parameters, can vary with size of polymer as well as conditions.

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Chain Branching in Poly-β-alanine[1,2]

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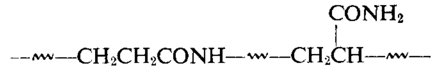
ABSTRACT: Proton magnetic resonance and infrared absorption studies have been carried out on poly-β-alanines prepared by *t*-butoxide-initiated polymerization of acrylamide in pyridine, nitrobenzene, dimethyl sulfoxide, and dioxane solutions in the presence of phenyl-β-naphthylamine free-radical inhibitor. The water-soluble fractions were found to be branched copolymers of β-alanine and β,β′-iminodipropionic acid with primary amide end groups. Carboxyl end groups were detected as well, possibly resulting from the hydrolysis of primary amide groups. These carboxyl groups were the probable cause of a polyelectrolyte expansion effect in viscosity studies of a salt-free aqueous solution of a highly branched sample (PBA-S1) at pH 6. Flow birefringence studies showed that PBA-S1 consisted of readily orientable, nonspherical molecules in aqueous solution, suggesting that relatively short branches occur along a rather extended "backbone." The water solubility of the polymers increased with increasing branching and decreasing molecular weight. It is suggested that branch growth is initiated during polymerization by the formation of secondary amide anions on the polymer chain.

The *t*-butoxide-initiated polymerization of acrylamide in the presence of a free-radical inhibitor such as phenyl-β-napthylamine or hydroquinone in an inert solvent has been reported to yield water-soluble and water-insoluble poly-β-alanine (reaction 1).[3-5] In preparation for a study of the conformation

$$n\mathrm{CH_2{=}CHCONH_2} \longrightarrow \mathrm{-(CH_2CH_2CONH)}_n\mathrm{-} \qquad (1)$$

of poly-β-alanine in aqueous solution,[6] we sought to determine directly the structure of the water-soluble samples.

Other investigators have noted from ir spectra that some of the products of reaction 1 contain anomalously high proportions of primary amide groups.[7-10] They suggested that these may arise from copolymers of polyacrylamide and poly-β-alanine, I.



$$\mathrm{-\!\sim\!-CH_2CH_2CONH-\!\sim\!-CH_2\overset{\overset{\displaystyle CONH_2}{|}}{C}H-\!\sim\!-}$$

I

We had been informed by Dr. M. Sloan, who supplied one of our samples, that β,β′-iminodipropionic acid was one of the hydrolysis products of polymers obtained by reaction 1. The pmr evidence reported here confirms this as well as the presence of anomalously high quantities of primary amide groups, and further shows that the primary amide groups are correlated with

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