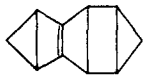
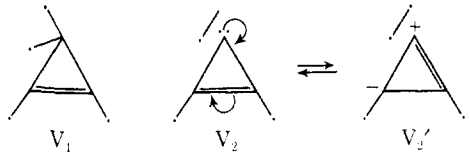
According to this method, 89% of the active π charge in benzene is represented by the two Kekulé structures: the remainder must be described by Dewar structures, implying some cross-ring binding. Only 81% of the π charge in azulene is accounted for by Kekulé structures. The Dewar form



is quite important; over 10% of the active π charge is involved in cross-ring bonding.

Systems expected to be highly localized are also unambiguously characterized by this method. The familiar structure of ethane embraces 99.5% of the active charge, according to the bond index analysis of the CNDO–MO function. The remainder of the active charge is involved in H–H bonding.

The most interesting use of the bond index analysis is the diagnosis of delocalization (and hence, nonclassical character) in carbonium ions and strained-ring systems. We give a single example here, reserving others for more leisurely discussion. In cyclopropene, 97% of the active charge is represented by the usual valence bond structure V_1. About two-thirds of the remaining active



charge is delocalized in the plane of the ring ("σ delocalization") and one-third is "π delocalized." The latter is predominantly due to a structure V_2 in which the apical protons are bonded, and the resulting carbon lone pair can roam about the ring. The σ delocalization involves a number of open-ring structures.

According to these examples, the bond index can be used to analyze the charge distribution into intuitively significant structures with, insofar as the wave function is accurate, meaningful numerical weights. This device should increase our understanding of MO treatments of molecules.

Acknowledgment. I wish to thank Professor O. Sinanoğlu for reading the manuscript and helpful suggestions. Thanks go to one of the referees for pointing out the significance of ionic structures in a MO wave function. This work was supported by a National Science Foundation postdoctoral fellowship.

(6) Present address: Chemistry Division, Argonne National Laboratory, Argonne, Ill. 60439

Carl Trindle[6]
Sterling Chemistry Laboratory, Yale University
New Haven, Connecticut
Received June 3, 1968

The Circular Dichroism of β-Poly-L-lysine[1]

Sir:

Recently, reports have appeared upon the optical rotary dispersion (ORD)[2,3] and circular dichroism (CD)[3,4] of poly-L-lysine $(Lys)_n$ solutions which had been heated to 50° at alkaline pH before analysis. Theoretical and experimental considerations suggest that $(Lys)_n$ solutions treated in such a manner are in the pleated β conformation.[2-7] Such data have therefore been used as a basis for evaluating the β structure present in proteins.[5,8] However, up to the present time, such attempts have been unsuccessful.

The minimum in the CD of previously heated $(Lys)_n$ solutions at around 216 mμ coincides with those of the polarized spectra of oriented $(Lys)_n$ films[7] which have been shown by uv and infrared spectroscopy as well as by X-ray diffraction[9] to be in the β conformation. However, different values in the magnitude of the molar residue ellipticity at the minimum have been reported, ranging from −19,000[4] to −23,000[3] deg cm²/dmol. Furthermore, kinetic experiments[2] and ultracentrifugational[3] analyses of the heated $(Lys)_n$ solution have led to different conclusions concerning whether the β structure is within or between polypeptide chains.

In our laboratory, difficulties were encountered in analyzing the CD of lens α-crystallin[10] by the method of curve fitting,[11] using $(Lys)_n$ heated to 50° at pH 11.5 as the reference for β structure. Because of such difficulties, an investigation of the effect of heating upon the $(Lys)_n$ was undertaken.

Curve 1 in Figure 1 represents the CD between 200 and 250 mμ of a heated solution of $(Lys)_n$ (LY-87-10, New England Nuclear Corp., Boston Mass.), prepared in *exactly* the same manner as reported by Greenfield, *et al.*[2] Based on such data, a mean molar residue ellipticity $[\theta]$ ranging from -16×10^3 to -25×10^3 deg cm²/(dmol) could be calculated. Amino acid analysis after hydrolysis in 6 *N* HCl indicated the presence of no other amino acid. Before heating, the sample appeared homogeneous by equilibrium centrifugation, giving a molecular weight of 40,700 ± 1200. However, after heating, a molecular weight could no longer be determined since a fine suspension had formed. This was demonstrated in the following manner. The heated solution, after cooling to room temperature, was immediately centrifuged in a Spinco Model L centrifuge at 26,400*g* (20,000 rpm) for 15 min at 20°. The CD of the supernatant (curve 3 of Figure 1) shows a typical double minima of an unheated $(Lys)_n$ solution at pH 11. Thus, the soluble $(Lys)_n$ appears to be in the α-helical form. By comparing the magnitude of the ellipticity of the supernatant with that of the original alkaline solution prior to heating, the amount of soluble $(Lys)_n$ left in the supernatant is about 12%. Resuspending the precipitate in alkaline KOH yielded curve 2 of Figure 1, again characteristic of the β conformation. This suspension was then fil-

(1) This research was supported by grants from the National Institute of Neurological Diseases and Blindness and the John Hartford Foundation. The technical assistance of Messrs. R. Kidwell, P. Rommel, and C. Chang is gratefully acknowledged.

(2) W. Greenfield, B. Davidson, and G. D. Fasman, *Biochemistry*, **6**, 1616 (1967).
(3) P. K. Sarkar and P. Doty, *Proc. Natl. Acad. Sci. U. S.*, **55**, 981 (1967).
(4) R. Townend, T. F. Kumosinski, and S. N. Timasheff, *Biochem. Biophys. Res. Commun.*, **23**, 163 (1966).
(5) N. Greenfield, B. Davidson, and G. D. Fasman, *Biochemistry*, **6**, 1630 (1967).
(6) E. S. Pysh, *Proc. Natl. Acad. Sci. U. S.*, **56**, 825 (1966).
(7) K. Rosenheck and B. Sommer, *J. Chem. Phys.*, **46**, 532 (1967).
(8) B. Jirgensons, *J. Am. Chem. Soc.*, **89**, 5980 (1967).
(9) E. R. Blout and H. Lenormant, *Nature*, **179**, 960 (1957).
(10) L. K. Li and A. Spector, *J. Biol. Chem.*, **242**, 3234 (1967).
(11) J. P. Carver, E. Shechter, and E. R. Blout, *J. Amer. Chem. Soc.*, **88**, 2562 (1966).

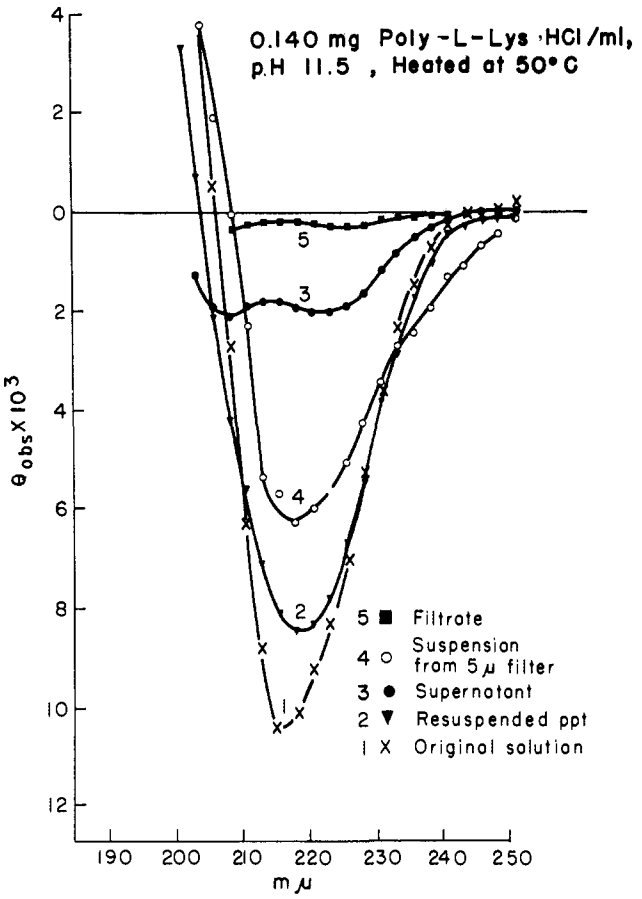


Figure 1. The CD of poly-L-lysine·HCl, heated at 50° at pH 11.5: 1, sample prepared by the procedure of Greenfield, *et al.*,[2] 2, suspension of sample 1 after centrifuging at 26,400*g* for 10 min; 3, supernatant of sample 1 after centrifuging; 4, resuspension of sample 2 after filtering through a 5-μ filter; 5, filtrate from sample 2.

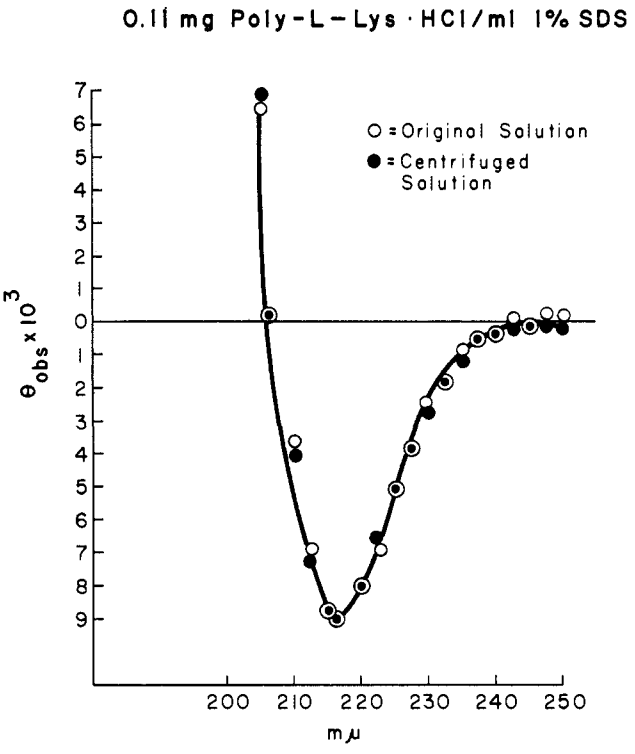


Figure 2. The CD of 0.11 mg of poly-L-lysine·HCl in 1% SDS. The original solutions (open circles) were measured immediately after passing through a 5-μ filter. The same solution, after centrifuging at 26,400*g* for 30 min, is represented by the filled circles.

tered through a Millipore filter with a pore size of 5.0 μ. The filtrate was almost indistinguishable from the base line (curve 5, Figure 1). The filter was then stirred in a beaker with the same volume of alkaline solution as the original suspension. Again, the β-type CD curve was obtained (curve 4, Figure 1). These experiments indicate that the precipitate had not been converted to the soluble α-helical form. No suggestion of an equilibrium between the two forms was noted over the time period of the experiments.

Since filtration can be accomplished within a few minutes, in some experiments the heated solution after cooling to room temperature was filtered immediately and the resuspended precipitate examined in the Cary 60 spectropolarimeter. Results similar to those described above were observed: *i.e.*, the typical β curve is always associated with the precipitate, and the supernatant contains approximately 15% of the material in the α-helical form. These experiments indicate that more than 85% of the $(Lys)_n$ aggregated to form particles greater than 5 μ in diameter after heating for 20 min at pH 11.2–11.7. Similar observations were made with several samples of $(Lys)_n$ with molecular weight varying from 12,000 to 61,000.

Since no filtration of the heated $(Lys)_n$ solutions was included in any published procedure for preparing the β form, it is likely that the reported CD[3,4] and ORD[2,4] were based upon observations of suspensions of similar aggregates, containing a small amount of α-helical material. Even though the positions of the dichroic bands are not shifted to a marked extent by insolubilization, the magnitude of the $[\theta]$ must be subject to uncertainty. Such aggregates can be considered as an infinite sheet of β structures. The relationship of sheet size to the rotational strength of the long-wavelength band of the β structure of poly-L-alanine has been calculated recently by Urry.[12] The results of such theoretical calculations show that, as the sheet becomes larger, the rotational strength of pleated β sheet becomes more negative. Certainly it does not appear possible to quantitatively evaluate the finite contribution of the β conformation to the dispersion of protein solutions from values obtained from suspensions (insoluble, infinite sheet) of $(Lys)_n$.

In order to circumvent the difficulty in solubilizing the β form, we have studied the CD spectra of $(Lys)_n$ in 1% sodium dodecyl sulfate. It has been shown by ultraviolet, infrared, as well as CD spectra that $(Lys)_n$ in this detergent assumes the β conformation.[3] Results obtained with $(Lys)_n$ (mol wt 41,000) under such conditions are shown in Figure 2. Unlike heated alkaline solutions of $(Lys)_n$, neither filtration nor centrifugation causes an appreciable change in the CD. The magnitude of the negative band at 216 mμ, $[\theta] = -9600$, is similar to the values obtained by Sarkar and Doty[3] as well as that observed for the β structure of silk fibroin in 93% methanol.[13]

The CD of several $(Lys)_n$ samples with different molecular weights were examined in 1% SDS. All gave curves similar to those shown in Figure 2. The

(12) D. W. Urry, *Proc. Natl. Acad. Sci. U. S.*, **60**, 394 (1968).
(13) E. Lizuka and J. T. Yang, *ibid.*, **55**, 1175 (1966).

Table I. Molar Ellipticities of the Various Conformations of Poly-L-lysine[a]

Mol wt[b] ± 5%	$-[\theta]_{196}$ ± 9% at pH 2–3[c]	$-[\theta]_{223}$ ± 11% at pH 11.5–11.7[d]	$-[\theta]_{216}$ ± 15% in 1% SDS
5,500	?	?	5.0×10^3
12,000	3.4×10^4	3.4×10^4	9.3×10^3
18,000	3.0×10^4	2.9×10^4	8.2×10^3
41,000	2.7×10^4	2.8×10^4	9.7×10^3
61,000	3.2×10^4	3.0×10^4	7.7×10^3

[a] Values are reported as mean molar residue ellipticities. The concentration was determined by the ninhydrin color after the sample had been hydrolyzed for 22 hr in 6 *N* HCl. The ninhydrin color was calibrated with a known L-lysine solution on the Technicon autoanalyzer. [b] Molecular weight determined by equilibrium ultracentrifugation. The high speed method of Yphantis (D. A. Yphantis, *Biochemistry*, **3**, 297 (1964)) was used with samples having molecular weights of 41,000 and 61,000. The remainder were determined with the low-speed method using either schlieren (K. E. Van Holde and R. L. Baldwin, *J. Phys. Chem.*, **62**, 734 (1958)) or interference (E. G. Richards and H. K. Schachman, *ibid.*, **63**, 1578 (1959)) optics. [c] The ellipticity was recorded directly in degrees on a Cary 60 spectropolarimeter with a Model 6001 CD accessory in either a 10-mm or a 1-mm cell. The signal to noise ratio is 10:1 or better over the wavelength range studied. [d] These values are found to be concentration dependent. Values included here were from solutions with a residue concentration of approximately 0.7 m*M*. Values of $[\theta]$ 25% lower were obtained with solutions of 0.07 m*M* residue concentration.

results are summarized in Table I. All samples of $(Lys)_n$ of mol wt 12,000 or greater gave $[\theta]$ at 216 mμ in the range -7.7×10^3 to -9.7×10^3 deg cm²/dmole. Thus, the absolute magnitude of the negative band decreases by approximately one-half to one-third as compared with that obtained from the previously heated $(Lys)_n$ in alkaline solution. The latter, as mentioned above, corresponds to an infinite sheet of β structure. Since the $(Lys)_n$ in SDS is soluble, it must therefore be a finite sheet size. This is confirmed by noting that all $(Lys)_n$ samples in SDS pass through a 0.22-μ pore-sized filter. As described before, the heated $(Lys)_n$ in an alkaline solution cannot pass through a filter having a pore size of 5 μ. The *change* in ellipticity as a function of sheet size agrees with the *empirical* calculation, which suggests a decrease in the *magnitude* of rotatory power as the sheet size becomes smaller.[12]

Since the $[\theta]$ values obtained with $(Lys)_n$ chains of 75–380 residues in length (mol wt 12,000–61,000) remain almost constant in SDS, and considering the calculated as well as the observed dependence of the ellipticity on the size of the β sheet, it follows that $(Lys)_n$ chains in this range of chain length do not produce a β structure of significantly different sheet size. This can only be accomplished by intrachain interaction, rather than interchain reaction, leading to the formation of a larger pleated sheet. The lack of concentration dependence in the values of $[\theta]_{216}$ in all the $(Lys)_n$ samples of varying chain lengths is also in support of this interpretation.

Further decreases in chain length from 75 to about 35 residues (mol wt 5500) cause further decreases in the absolute magnitude of the negative CD band and in the rotary power by about 40% (Table I) without changing the characteristics in the β-type CD curve. However, at a chain length of 20 residues (mol wt 3300) the typical β-type CD cannot be formed by the addition of 1% SDS. Apparently a minimal chain-length requirement must be met in order to form this type of intrachain pleated sheet of $(Lys)_n$ in SDS. This minimum lies somewhere between 20 and 35 residues. Longer chains, up to 75 residues, produce longer sheets, characterized by an increase in rotational strength of the long-wavelength transition. Beyond this chain length, further increases in ellipticity cannot be demonstrated, as mentioned above.

Comparative studies were also carried out with the random coil and the α-helical form of $(Lys)_n$. The $[\theta]$ at 196 mμ at pH 2–3 for the random coil form and the $[\theta]$ at 223 mμ at pH 11.5 for the α-helical form are also included in Table I. The double minima feature at 208 and 223 mμ in the CD of $(Lys)_n$ at alkaline pH have been considered to be characteristic of an α helix. This feature was produced only by $(Lys)_n$ samples of mol wt 12,000 (75 residues) or greater. The sample of 35 residues (mol wt 5500) does not give the double minima CD when the pH of the solution was adjusted to 11.5. Instead, a strong negative dichroic band at 202 mμ became dominant. The ellipticities of all samples giving the double minima feature show concentration dependence. This suggests a repeating structure of the peptide backbone resulting from *interchain* interaction rather than that of an α helix. Under conditions for obtaining the random coil, $(Lys)_n$ samples of 35 residues or less in length demonstrated a much broader trough around the 196-mμ region, and the magnitudes of the $[\theta]$ at 196 mμ were considerably smaller than the values obtained with higher molecular weight material. These complications indicate that great caution must be exercised in utilizing CD data of $(Lys)_n$ for interpreting the conformation of protein solutions.

Lu-Ku Li, Abraham Spector

Department of Ophthalmology, College of Physicians and Surgeons Columbia University, New York, New York 10032

Received June 5, 1968