four-coordinated, S-bonded Cu(III) complex with a 1,1-dithio chelate (2.22 (2) Å in $Cu(Dtc)_2I_3$). A comparison of the Cu–S bond lengths in $Cu(n\text{-}Bu_2Dtc)_2I_3$ to those in $Cu(Et_2Dtc)_2$[11] shows that changes in bond lengths within the dithiocarbamate ligand are not significant, and the only real difference is found in the Cu–S bond (shorter by ~0.08 Å in the planar, diamagnetic Cu(III) complex). Recent epr studies have indicated[12] that the unpaired electron in $Cu(Dtc)_2$ resides in a σ antibonding orbital which is mainly composed of metal and sulfur orbitals. Removal of this electron would be expected to increase the strength of the Cu–S bond. Similar reasoning, involving a high energy σ antibonding orbital,[13] most probably accounts for the remarkably low oxidation potential of the $Cu(DED)_2^{2-}$ complex.

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(11) M. Bonamico, G. Dessy, A. Mugnoli, A. Vaciago, and L. Zambonelli, *Acta Crystallogr.*, **19**, 886 (1965).
(12) M. J. Weeks and J. P. Fackler, *Inorg. Chem.*, 7, 2548 (1968).
(13) A typical four-line epr spectrum was observed in a CH_2Cl_2 solution of I. A value for $\langle g_{eff} \rangle$ of 2.043 ± 0.001 was found with $\langle A \rangle$ = 75.7 G. $\langle g_{eff} \rangle$ values of 2.043–2.045 have been reported[6] for other copper(II) dithiolates.
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Conformation and Optical Properties of Poly(L-valine) in Aqueous Solution. "A Single Extended β Chain"[1]

Sir:

The optical properties of the β conformation of polypeptides are not yet well understood due to complexity dependent on the multitude of molecular states determined by the number of associated pleated sheets and their chain length, despite the fact that several detailed theoretical studies[2–5] have been carried out. A major distinction of the β conformation, relative to the α-helical form, is that the molecular state, *i.e.*, the number of associated chains,[2–4] severely influences the optical properties. Thus, all the attempts[6–8] to predict protein conformation based on chiroptical properties are still rather arbitrary, as different sets of standard circular dichroism patterns of helical, β, and random conformations are used. In this paper, we report the optical properties of a species of poly(L-valine) which is probably a single extended chain β structure. To solubilize this polymer in aqueous solution, it was flanked with DL-lysyl·HCl blocks on both sides yielding poly(DL-Lys)$_{100}$-poly(L-Val)$_{200}$-poly(DL-Lys)$_{100}$ [(DL-Lys)(L-Val)-(DL-Lys)], a typical sandwich polymer as introduced by Gratzer and Doty.[9] This polymer was synthesized by polymerizing the corresponding amino acid–*N*-carboxyanhydrides successively in dimethylformamide using *n*-hexylamine as an initiator.[10] The actual molar ratio of each residue in the copolymer as determined by amino acid analysis was Lys 57.2% and Val 42.8%.

The circular dichroism (CD) spectra and ultraviolet absorption spectra of (DL-Lys)(L-Val)(DL-Lys) in water (pH 7) are shown in Figure 1. These measurements were made respectively with a Cary 60 spectropolarimeter with a CD attachement (No. 6001) and a Cary 14 (under a nitrogen atmosphere).

The CD extrema are seen at 195 nm (peak $[\theta]_{195}$ = 16,000) and at 215–216 nm (trough $[\theta]_{215}$ = −25,000) where $[\theta]$ is expressed in molar ellipticity (deg cm^2 dmol^{-1}). The uv spectrum showed the maxima at 192 nm with ϵ (molar extinction coefficient) 10,600, after correction for the DL-lysyl block based on absorption spectra of poly(DL-Lys)$_{25}$. As poly(DL-Lys)$_{25}$, in the random conformation at pH 7.0, had ϵ_{max}^{192} 6200, this new form had a 70% relative hyperchromicity. This hyperchromicity can be attributed to the β conformation[11] of the central valyl block. To confirm this conformational assignment, infrared spectroscopy in D_2O solution was carried out as shown in Figure 2.

Poly(DL-Lys·HCl)$_{25}$ shows the amide I′ band (D_2O) at 1645 cm^{-1}, characteristic of the random form.[12] (DL-Lys)(L-Val)(DL-Lys) has a sharp amide I′ peak (D_2O) at 1636 cm^{-1} and a weak shoulder in the 1650-cm^{-1} region. As the 1650-cm^{-1} shoulder can be attributed to the random lysyl block, the 1636-cm^{-1} band is due to the L-valyl block. The characteristic amide I′ of helical synthetic polypeptides is observed at *ca.* 1638 cm^{-1} [12] and thus the 1636-cm^{-1} band would suggest the helical conformation of the valyl block. However, it should be noted that the characteristic amide I′ frequency of the β form in proteins differs from that of synthetic polypeptides,[12] and is seen at 1632 cm^{-1} for proteins while found at 1611 cm^{-1} for synthetic polypeptides. It can be expected that the valyl block will be surrounded by hydrocarbon side chains of the charged random DL-lysyl blocks rather than with water because of the strong hydrophobic character of the valyl residue. Therefore, the environment around the valyl block will be similar to that of certain segments in the interior of proteins. Thus, infrared results obtained on proteins will be more applicable for the conformational assignment of the valyl block. Thus from the infrared bands, the CD extrema, and the large hyperchromicity, the β conformation of the valyl block is unequivocally assigned. This conformational assignment was also supported by far-infrared solid state studies which indicated that there were no valyl residues in the helical conformation.[10] It is interesting to note the absence of the band at *ca.* 1680 cm^{-1}, characteristic of the antiparallel β form.[13] This band is absent and not arti-

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(2) E. S. Pysh, *Proc. Nat. Acad. Sci. U. S.*, **56**, 825 (1966).
(3) R. W. Woody, *Biopolymers*, **8**, 669 (1969).
(4) V. Madison and J. Schellman, *Biopolymers*, **11**, 1041 (1972).
(5) M. V. Volkenstein and V. A. Zubkov, *Biopolymers*, **5**, 465 (1967); T. M. Birnshtein and O. B. Ptitsyn, *ibid.*, **5**, 785 (1967).
(6) N. Greenfield and G. D. Fasman, *Biochemistry*, **8**, 4108 (1969).
(7) V. P. Saxena and D. B. Wetlaufer, *Proc. Nat. Acad. Sci. U. S.*, **68**, 969 (1971).
(8) T. H. Chen, J. T. Yang, and H. M. Martinez, *Biochemistry*, **11**, 4120 (1972).
(9) W. B. Gratzer and P. Doty, *J. Amer. Chem. Soc.*, **85**, 1193 (1963).
(10) S. Kubota and G. Fasman, to be submitted for publication.
(11) K. Rosenheck and P. Doty, *Proc. Nat. Acad. Sci. U. S.*, **47**, 1775 (1961).
(12) H. Susi, S. N. Timasheff, and L. Stevens, *J. Biol. Chem.*, **242**, 5460 (1967).
(13) T. Miyazawa and E. R. Blout, *J. Amer. Chem. Soc.*, **83**, 712 (1961).

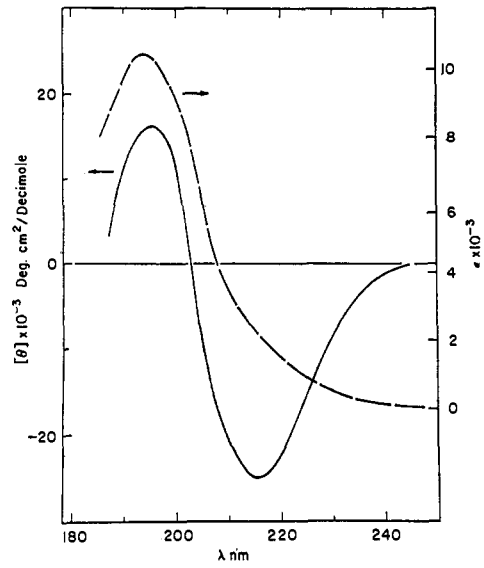

Figure 1. Circular dichroism and ultraviolet spectra of poly(DL-Lys·HCl)$_{100}$(L-Val)$_{200}$(DL-Lys·HCl)$_{100}$ in water at pH 6.78: (—) CD, (- - -) uv. Concentration of polymer is ≈0.02%, path length of cells, 1–5 mm.

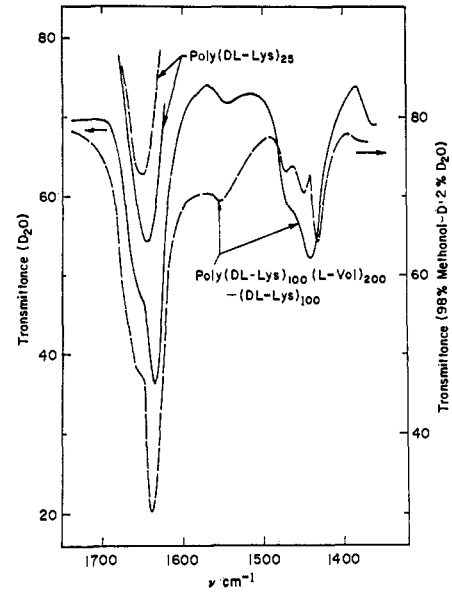

Figure 2. Infrared spectra of poly(DL-Lys·HCl)$_{100}$(L-Val)$_{200}$(DL-Lys·HCl)$_{100}$ and poly(DL-Lys·HCl)$_{25}$: D$_2$O (—); 98% MeOD–2% D$_2$O (- - -). Concentration of polymers is 2–3%, path length of cells 0.05–0.01 mm.

ficially lost due to the overlapping of bands, as the 1680-cm^{-1} band is usually quite sharp in solution compared to the 1650-cm^{-1} band. The homopolymer, poly(L-valine), assumes the antiparallel pleated β form.[14–17] Thus the valyl block in the block copolymer might assume the antiparallel β form if intra- or interchain association were not prohibited by either the high charge density of the lysyl blocks or the complete insulation of the valyl block by association to the lysyl blocks. To illustrate this lack of valyl chain association, it was found that the CD spectrum was completely independent of polymer concentration (up to ≈0.2%). In addition, the CD spectrum of the polymer (at ≈0.02%) was independent of salt concentration (NaCl) up to 0.5 *M* where opalescence took place. This evidence indicates that the valyl blocks are well isolated from each other and the association between them is prohibited by the lysyl blocks as discussed above. Direct evidence for a single chain β was not obtainable because of (a) the polyelectrolyte behavior in absence of salts, *i.e.*, increase in $\eta sp/c$ on reducing the ionic strength and precipitation of the polymer at high salt (>0.05 *M* NaCl, polymer concentration >0.08%) where the polyelectrolyte behavior would be eliminated and (b) sedimentation studies were prohibited for similar reasons.

The magnitude at the minima (n–π* transition) and at the maxima (π–π* transition) is the largest and smallest, respectively, of all the reported values for β-polypeptides. According to theoretical considerations,[3,4] the magnitude of the n–π* transition increases while the magnitude of the π–π* transition decreases on reducing the number of associated pleated sheets. It has been found[10] that the chain length dependence of the CD spectra of this β conformation is rather small.

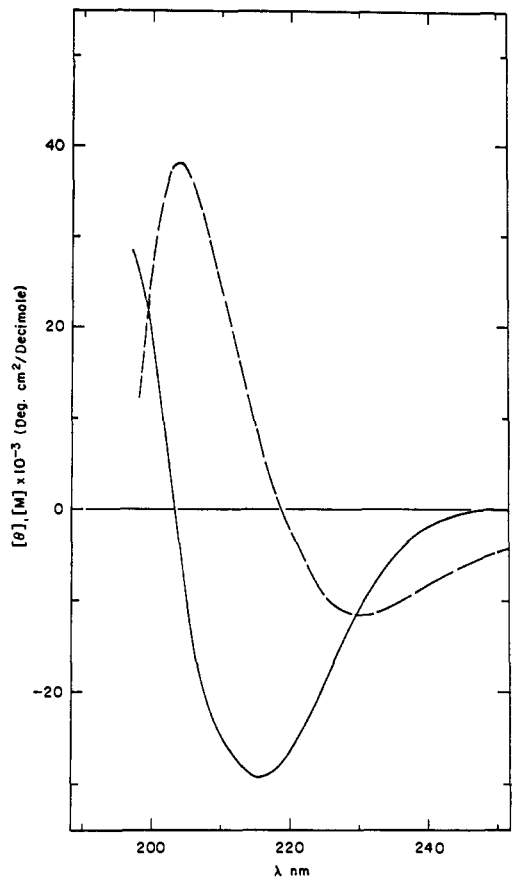

Figure 3. Circular dichroism and optical rotatory dispersion spectra of poly(DL-Lys·HCl)$_{100}$(L-Val)$_{200}$(DL-Lys·HCl)$_{100}$ in 98% MeOH–2% H$_2$O: (—) CD, (- - -) ORD. Concentration of polymer is ≈0.03%, path length of cells, 0.5–5 mm.

Therefore, the valyl block examined therein will consist of a minimum number of pleated sheets and probably is only a single extended β chain, because of the absence of the 1680-cm^{-1} band as discussed above.

(14) E. R. Blout, C. de Loze, S. M. Bloom, and G. D. Fasman, *J. Amer. Chem. Soc.*, **82**, 3784 (1960).
(15) S. J. Leach, G. Nemethy, and H. A. Scheraga, *Biopolymers*, **4**, 369 (1966).
(16) J. L. Koenig and P. L. Sutton, *Biopolymers*, **10**, 89 (1971).
(17) D. B. Fraser, B. S. Harrap, T. P. MacRae, F. H. C. Stewart, and E. Suzuki, *J. Mol. Biol.*, **12**, 482 (1965).

Epand and Scheraga[18] previously reported that a similar polymer, poly(DL-Lys)$_{18}$(L-Val)$_{15}$(DL-Lys)$_{16}$, underwent a conformational transition from β in water to α-helix by the addition of methanol (98%). The observed ellipticity was quite small compared to the values reported herein. Our results are in conflict with this report as shown in Figures 2 and 3.

The infrared spectrum in 98% methanol is nearly identical with that found in water and no conformational change can be detected. Similarly, in Figure 3, a single negative trough is seen at 215–216 nm with $[\theta] = -29{,}400$ with a cross-over point at $\sim$203 nm. The ORD showed a trough at 230 nm ([M] refers to mean residue molar rotation) and a peak at 204 nm ([M] = 38,500). Both these spectra are characteristic of the β form[19] and are similar to that found in H_2O (Figure 1). Therefore, it is apparent that no conformational transition of β to α-helix occurred in the valyl block on changing the aqueous solvent to MeOH. Our results agree with the theoretical conformational calculations which indicate that the β form is the most preferred for the valyl residue[15,20,21] in contrast to other studies.[22] This report records the optical properties of a single extended β chain in contrast to previous reports of highly associated β sheets.

(18) R. E. Epand and H. A. Scheraga, *Biopolymers*, **6**, 1551 (1968).
(19) A. J. Adler, N. J. Greenfield, and G. D. Fasman, *Methods Enzymol.*, **27**, 675 (1973).
(20) P. K. Ponnuswamy and V. Sasisekharan, *Biopolymers*, **10**, 89 (1971).
(21) A. M. Liquori, *J. Polymer Sci., Part C*, **12**, 209 (1966); "Ciba Symposium on Principles of Biomolecular Organization," G. E. W. Wolstenholme and M. O'Conner, Ed., Little and Brown Co., Boston, Mass., 1966, p 40.
(22) T. Ooi, R. A. Scott, G. Vanderkooi, and H. A. Scheraga, *J. Chem. Phys.*, **46**, 4410 (1967).

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Nucleoside Complexing. Interligand Interactions between Purine and Pyrimidine Exocyclic Groups and Polyamines. The Importance of Both Hydrogen Bonds and Nonbonding Repulsions

Sir:

Selective attachment of heavy-atom-containing moieties to biopolymers should permit an electron microscopic study of their structure.[1] In particular, the sequence of bases in DNA might be determined if a heavy metal can be selectively bound to one of the four common heterocyclic bases. There are some differences in the coordinating affinity of a given metal ion for the common deoxynucleosides,[2] namely, deoxythymidine (dT), deoxycytidine (dC), deoxyadenosine (dA), and deoxyguanosine (dG). However, these nucleosides are unfortunately not sufficiently different in their coordinating tendencies to allow a meaningful labeling study.[3] Therefore, several alternative approaches to the induction of selectivity have been tried. These include: (1) specific reaction of a heavy-metal compound (OsO_4) with a base (thymine),[4] (2) formation of nucleic acids from covalently bound heavy-metal-labeled precursors (organomercurials[5] of purine and pyrimidine nucleotides), (3) specific organic reactions which introduce a functional group which is then available for binding by a metal ion or complex,[6] and (4) examination of nucleic acids which naturally contain a very few reactive bases (thiouracil).[7] There is presently a great need for a heavy metal label for A. Selective reagents, even those containing only light atoms, are useful in structural studies of nucleic acids.[8]

Recently, we have begun to explore a different approach to selective labeling.[9] We believe that both hydrogen-bonding and nonbonding repulsive interactions between the exocyclic group on the bases and the chelate ligands in metal complexes will lead to selective reactions. In essence, the propensity of *the nucleic acid constituent bases to recognize complementary bases via hydrogen-bonding interactions can be exploited in specific labeling schemes or in the development of specific reagents.*

We now wish to report our studies on the reaction of *cis*-β-$[Co(trien)Cl_2]^+$ (where trien = triethylenetetramine, $NH_2CH_2CH_2NHCH_2CH_2NHCH_2CH_2NH_2$) with ^{14}C-labeled dT, dC, dG, and dA. Our reasons for choosing this particular system were as follows. First, the nucleosides are extremely weak ligands,[2] especially toward octahedral complexes, and radiotracer techniques are necessary for detecting complex formation in the presence of excess complex reagent ($[Co(trien)Cl_2]^+$). Second, we have previously studied a complex of the purine theophylline (1,3-dimethyl-2,6-dioxopurine).[9] The structure of this complex, *trans*-$[Co(en)_2Cl(theophyllinato)]^+$ (en = ethylenediamine, $NH_2CH_2CH_2NH_2$), revealed that the amino hydrogens of the ethylenediamine ligands are capable of donating a hydrogen bond to the exocyclic oxygen on C(6) of theophylline. This purine was coordinated *via* N(7), as expected of dG. The trien system offers essentially the same hydrogen-bonding potential as the ethylenediamine complexes. Although preparatively more attractive, the bis(ethylenediamine) complexes usually produce more by-products and react more slowly than the analogous trien complexes.[10]

A schematic representation of the possible interactions between the nucleoside bases and the NH and/or NH_2 groups of the coordinated triethylenetetramine ligand is given in Figure 1. Thymine (deprotonated at N(3)) will probably coordinate *via* N(3). The two exocyclic oxygens (at C(2) and C(4)) are then in a favorable position to hydrogen bond to the NH_2 or NH groups of the trien chelate (Figure 1A). The N(3) in the cytosine

(1) J. W. Wiggins and M. Beer, *Anal. Chem.*, **44**, 77A (1972).
(2) G. L. Eichhorn, "Inorganic Biochemistry," G. L. Eichhorn, Ed., Elsevier, Amsterdam, 1973, Chapter 33.
(3) A claim that alkaline earth metal ions react selectively with guanosine has been shown to be incorrect: C. H. Chang and L. G. Marzilli, *J. Amer. Chem. Soc.*, **96**, 3656 (1974).
(4) P. J. Highton, B. L. Murr, F. Shafa, and M. Beer, *Biochemistry*, 7, 825 (1968).
(5) M. K. Dale, D. C. Livingston, and D. C. Ward, *Proc. Nat. Acad. Sci. U. S.*, 70, 2238 (1973).
(6) E. N. Moudrianakis and M. Beer, *Proc. Nat. Acad. Sci. U. S.*, **53**, 563 (1965).
(7) H. I. Heitner, S. J. Lippard, and H. R. Sunshine, *J. Amer. Chem. Soc.*, **94**, 8936 (1972).
(8) J. R. Barrio and N. J. Leonard, *J. Amer. Chem. Soc.*, **95**, 1323 (1973).
(9) T. J. Kistenmacher, L. G. Marzilli, and C. H. Chang, *J. Amer. Chem. Soc.*, **95**, 5817 (1973); L. G. Marzilli, T. J. Kistenmacher, and C. H. Chang, *ibid.*, **95**, 7507 (1973).
(10) D. A. Buckingham, J. P. Collman, D. A. R. Happer, and L. G. Marzilli, *J. Amer. Chem. Soc.*, **89**, 1082 (1967); S. K. Oh and C. B. Storm, *Bioinorg. Chem.*, **3**, 89 (1973).