relation of factorability to statistical binding, apply here as well as in the macroscopic thermodynamic case.

In the event that the binding of more than one type of ligand molecule to the protein is considered explicitly, the binding potential assumes the form

$$\Pi = kT \ln \left(\sum_{\mathbf{s}} \lambda_x^{s_x} \lambda_y^{s_y} \ldots H_{\mathbf{s}}/H_0 \right) \qquad (56)$$

where $\mathbf{s}$ refers to s_x, s_y, ... molecules of the different ligands, $H_{\mathbf{s}}$ is the effective partition function for a single molecule of protein to which $\mathbf{s}$ molecules of ligand are bound, and λ_i is the absolute activity of ligand species i. If $H_{\mathbf{s}}/H_0$ is interpreted as an equilibrium constant for the reaction

$$P_0 + s_x X + s_y Y + \ldots \qquad PX_{s_x} Y_{s_y} \ldots \qquad (57)$$

the polynomial in 56 becomes formally identical with the macroscopic binding polynomial for the case where the number of ligands is greater than one.[16]

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(16) See expression 20 of ref 3.

A Theoretical Study of the Optical Rotatory Properties of Poly-L-tyrosine[1]

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Abstract: The optical rotatory properties of poly-L-tyrosine (PLT) are very different from those of simple polypeptides, and the helix sense of PLT remains uncertain. Calculations of rotational strengths for peptide and side-chain transitions have been performed for four conformations of low potential energy. Two of these are right handed and two are left handed, the conformations of a given screw sense differing in side-chain conformation. The results of these calculations were shown to be qualitatively independent of chain length and of small variations in side-chain conformation. Comparison of calculated and experimental circular dichroism curves indicates that only one of the four conformations is consistent with experiment, and that is the conformation we have denoted as RA. We conclude that poly-L-tyrosine forms a *right-handed* helix. Calculations of Ooi, *et al.*, yield a minimum potential energy for a right-handed helix, but predict a different side-chain conformation (R1). We propose that the RA conformation is lower than the R1 in free energy because of greater side-chain flexibility and hence more positive entropy.

The optical rotatory properties of simple helical polypeptides are now well understood, and unambiguous assignments of helix sense can be made on the basis of optical rotatory dispersion[2] (ORD) or circular dichroism[3] (CD). By simple polypeptides, we mean those which have alkyl side chains, side chains with weak chromophores (carboxyl, ester, or amino groups), or aromatic groups beyond the γ carbon (*e.g.*, poly-γ-benzyl-L-glutamate). Polypeptides such as poly-L-tyrosine (PLT) and poly-L-phenylalanine, which have aromatic rings attached to the β carbon, have drastically altered optical rotatory properties, and the helix sense remains uncertain.[4]

The ORD behavior of PLT in the visible region was observed to be anomalous.[5,6] Fasman[7] showed that copolymers of L-tyrosine and L-glutamic acid of varying composition showed a smooth and nearly linear change in the Moffitt–Yang[8] b_0 parameter from *ca.* +500, characteristic of PLT, to *ca.* −600, characteristic of poly-L-glutamic acid (PGA). This indicated that L-tyrosine residues fit into the right-handed PGA helix and implied but did not conclusively prove that PLT forms a right-handed α helix.

Subsequently, Fasman *et al.*,[9] extended ORD measurements on PLT down to 227 nm and Beychok and Fasman[10] reported the CD spectrum in the 214–300-nm region. The negative Cotton affect at 224 nm was assigned to the peptide n–π^* transition, and because its sign coincided with that of the n–π^* Cotton effect in simple polypeptides in the right-handed α-helix conformation, a right-handed screw sense of the PLT helix was taken to be confirmed. However, this interpretation can be questioned. The amplitude of the 224-nm Cotton effect in PLT is only about one-third that of the 222-nm band in simple α-helical polypeptides.[3] This reduction in magnitude was attributed[10] to band overlap and/or coupling with transitions characteristic of the phenolic side chain. It is possible that coupling with

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(1) (a) Based in part on the Ph.D. Thesis of A. K. Chen, University of Illinois, Urbana, Ill., 1969; presented in part at the 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969, Abstract BIOL 275.

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side-chain transitions may not only alter the *magnitude* of the n–π^* Cotton effect, but even its *sign*.

Pao, *et al.*,[11] carried out a theoretical treatment for PLT in the α-helical conformation. This treatment focused attention on the exciton interactions[12] of the peptide n–π^* and π–π^* transitions and of the two long-wavelength phenolic transitions centered at about 277 and 225 nm. The two fully allowed phenolic transitions at about 190 nm were neglected, as was the mixing of nondegenerate states. Not unexpectedly, the exciton interaction in the 277- and 225-nm phenolic bands and the peptide n–π^* band turned out to be very small. Although the results of Pao, *et al.*, for a right-handed helix showed rough agreement with the ORD data of Fasman, *et al.*,[9] the omission of essential features of the phenolic side chains suggests that the agreement is largely fortuitous.

Applequist and Mahr[13] measured the dipole moment per residue of PLT and of its bromo derivative in dilute solution in quinoline. From space-filling models, they deduced favored side-chain conformations for right- and for left-handed helices. They then calculated the change in dipole moment ($\Delta\mu$) to be expected on bromine substitution, assuming no conformational change on bromination. Right- and left-handed helices gave opposite signs for $\Delta\mu$, and their experimental results agreed in sign and approximate magnitude with those calculated for left-handed helices. Thus they arrived at a helix sense opposite to that inferred from optical rotatory properties. Applequist and Mahr pointed out several of the defects in the optical rotatory approach which we have previously mentioned.

Conformational energy calculations on PLT have been reported by Ooi, *et al.*,[14] and Yan, *et al.*[15] These calculations indicate that a right-handed helix has the minimum potential energy, lower by 0.4–1.8 kcal/residue than the most stable left-handed helix. Ooi, *et al.*, were able to reconcile their results with the data of Applequist and Mahr.[13] The right-handed helical conformation which Ooi, *et al.*, predicted to be most stable had a side-chain conformation substantially different from that assumed by Applequist and Mahr. When the effect of bromine substitution on the dipole moment of this conformation was calculated, it agreed with experiment in sign, though not as well in magnitude as Applequist and Mahr's left-handed conformation.

The purpose of the present study was to carry out as complete a theoretical treatment of the optical rotatory properties of PLT as possible and to compare the predicted properties of several assumed conformations with available experimental data. Assuming that our present knowledge of optical rotatory theory and of the electronic properties of the amide and phenolic chromophores is adequate, it should be possible to eliminate the previous ambiguities in the interpretation of the ORD and CD of PLT, to assign the helix sense, and perhaps to obtain information on the side-chain orientation. We believe that we have succeeded in these goals.

Methods

In order to calculate the optical properties of a molecule, it is necessary to have a well-defined geometry. In this work, we have considered four α-helical conformations of PLT. Two of these, R1 and L1, are, respectively, the right- and left-handed α helices of minimum potential energy obtained from the calculations of Scheraga and coworkers.[14,15] The other two, RA and LA, represent conformations with locally minimal potential energy,[14,15] having side-chain conformations similar to those assumed by Applequist and Mahr[13] for, respectively, right- and left-handed helices. The main difference between the two types of conformation of a given screw sense is in the conformation about the C_β–C_α bond described by the dihedral angle χ_1 in the standard notation introduced by Edsall, *et al.*[16]

The dihedral angles φ, ψ, χ_1, and χ_2 describing the four conformations considered are given in Table I. The coordinate transformations described by Ooi, *et al.*,[14] and by Sugeta and Miyazawa[17] were combined to convert a given set of dihedral angles into a cylindrical coordinate system with the helix axis as the z axis, and to calculate the unit height, h, and unit twist, t, characterizing the helix. The parameters h and t are also given in Table I for the four conformations. Successive application of the basic screw operation can be used to generate the coordinates of all the atoms in the helix from those in a reference residue.

Table I. Dihedral Angles and Helix Parameters for PLT Conformations

	Dihedral angles, deg				Unit height, Å	Unit twist, deg
	φ	ψ	χ_1	χ_2	h	t
R1[a]	130.6	124.1	302.9	155.9	1.4719	98.58
RA[b]	130.3	123.1	171.5	273.4	1.4588	98.07
L1[a]	228.7	237.2	171.2	225.8	−1.4757	98.39
LA[b]	229.7	236.9	306.0	85.4	−1.4588	98.07

[a] See ref 15. [b] Private communication from H. A. Scheraga.

One must also specify the orientation of the phenolic OH group. Since the phenolic group is assumed to be planar, this OH may have two orientations, corresponding to χ_2 values differing by 180°. Scheraga and coworkers[14,15] found that the effect of the orientation of the OH group on the potential energy is quite small.

Our calculation of rotational strengths follows the Kirkwood–Moffitt–Tinoco approach,[12,18–20] incorporating some modifications used by Woody[21] in treating simple polypeptides. Similar modifications have been made by Schellman and coworkers using a matrix formalism.[22] In PLT we have two chromophores in each residue, the amide group and the phenolic group. It is the coupling between excited states

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of these groups which gives rise to optical activity. If $\psi_{i\alpha}$ represents the excited state α in the residue i, we can represent an excited state of an N-mer, allowing for coupling, by the wave function

$$\psi_k = \sum_{i=1}^{N} \sum_{\alpha=1}^{M} C_{ki\alpha}\psi_{i\alpha} \tag{1}$$

where M is the number of excited states considered in each residue, and $C_{ki\alpha}$ is a coefficient describing the degree of participation of the excited state α in the ith residue in the excited state ψ_k. There will be MN such excited states for the N-mer.

The coefficients $C_{ki\alpha}$, and energies E_k, of the excited states ψ_k are obtained by solving the secular determinantal equation

$$|V_{i\alpha;j\beta} - \delta_{i\alpha;j\beta}E| = 0 \tag{2}$$

Here $\delta_{i\alpha;j\beta}$ is the Kronecker δ. If $i\alpha = j\beta$, $V_{i\alpha;j\beta} = E_\alpha$, the energy of the excited state α in the isolated residue with respect to the ground state of the residue. If $i \neq j$, $V_{i\alpha;j\beta} = V_{i0\alpha;j0\beta}$, the energy of interaction between the transition charge densities for the appropriate transitions in the different residues. If $i = j$, but α and β are excited states of different groups within the residue (*e.g.*, α is an amide excited state while β is a phenolic excited state), then $V_{i\alpha;i\beta} = V_{i0\alpha;i0\beta}$. However, if $i = j$ and α and β are excited states of the same group, then $V_{i\alpha;i\beta} = \Sigma_{j\neq i}V_{i\alpha\beta;j00} + V_{i\alpha\beta;i'00}$, the interaction energy of the transition charge density between the excited states α and β and the permanent dipole field of the rest of the molecule. The term $V_{i\alpha\beta;i'00}$ indicates that the permanent dipole field of the other groups within the ith residue must be included, excluding only the group in which the excited states α and β are localized.

From the wave functions ψ_k for each excited state, the electric and magnetic dipole transition moments, $\boldsymbol{\mu}_k$ and $\mathbf{m}_k$, respectively, connecting the excited state to the ground state can be calculated

$$\boldsymbol{\mu}_{0k} = \sum_{j=1}^{N} \sum_{\alpha=1}^{M} C_{kj\alpha}\boldsymbol{\mu}_{j\alpha} \tag{3}$$

$$\mathbf{m}_{k0} = \sum_{j=1}^{N} \sum_{\alpha=1}^{M} C_{kj\alpha}\left\{\mathbf{m}_{j\alpha} + \frac{\pi i E_\alpha}{hc}\mathbf{R}_{j\alpha} \times \boldsymbol{\mu}_{j\alpha}\right\} \tag{4}$$

where $i = \sqrt{-1}$, $\boldsymbol{\mu}_{j\alpha}$ and $\mathbf{m}_{j\alpha}$ are the electric and magnetic dipole transition moments, respectively, for the transition from the ground state of residue j to the excited states α, and $\mathbf{R}_{j\alpha}$ is the position of the "center" of the corresponding localized transition.

Finally, one can calculate the rotational strength of the transition from the ground state of the polymer to each of the MN excited states

$$R_k = \mathrm{Im}\{\boldsymbol{\mu}_{0k}\cdot\mathbf{m}_{k0}\} \tag{5}$$

The interaction energies, $V_{i\alpha;j\beta}$, which enter into the secular determinant are calculated using a monopole–monopole approximation,[19] in preference to a dipole–dipole or quadrupole–dipole approximation. This corresponds to distributing the dipole or quadrupole describing the transition charge density. Some uncertainty is introduced by this approach because approximate wave functions must generally be used to calculate the monopole positions and charges, but it is felt that this defect is more than compensated for by the fact that the dipole–dipole approximation fails at small intergroup distances.

For the amide transitions, we considered only the n–π^* and the lowest energy π–π^* transition, since higher energy transitions are relatively unimportant in the wavelength region above 180 nm which is to be considered. The monopole positions and charges, the location of the center of the amide group, and the n–π^* magnetic dipole transition moment were the same as those used by Woody[21,23] in recent work on simple polypeptides.

The electronic structure of the phenolic side chain is taken to be the same as that of phenol. Self-consistent-field molecular orbital calculations on phenol have been carried out by Nishimoto and Fujishiro[24] and by Forsen and Alm.[25] Kimura and Nagakura[26] measured the vacuum-ultraviolet spectrum of phenol vapor in the wavelength range from 155 to 220 nm, and also carried out molecular orbital calculations. All three theoretical treatments give good agreement with Kimura and Nagakura's experimental results. We have used the wave functions obtained by Nishimoto and Fujishiro in this work.

Phenol has four transitions at wavelengths longer than 180 nm. The excited states involved in these transitions are analogous to the B_{2u}, B_{1u}, and E_{1u} states of benzene.[27] Tyrosine in neutral aqueous solution has absorption maxima at 275, 224, and 192.5 nm.[28] The latter band presumably contains two nearly degenerate bands analogous to the $A_{1g} \rightarrow E_{1u}$ benzene transition. We refer to these transitions, in order of increasing energy, as T1, T2, T3, and T4.

Table II shows the theoretical polarization direction, transition dipole moment, and oscillator strength calculated from Nishimoto and Fujishiro's wave functions for the four phenolic transitions.[24] Also shown are the experimental oscillator strengths taken from Kimura and Nagakura.[26] Kimura and Nagakura decomposed the intense 185-nm phenol band into two components of different oscillator strength, but the bands overlap so strongly that this resolution is not likely to be very accurate. Therefore we have assumed that the two components each have half of the total oscillator strength. In the case of the T1 transition, calculated and observed oscillator strengths agree well, and so monopole charges were calculated directly from the wave functions. The remaining three transitions show a more typical overestimate of oscillator strength by theory. We have therefore applied correction factors to the calculated monopole charges such that the observed and calculated oscillator strengths are brought into agreement. These factors are given in Table II and are 0.70–0.75. Also given in Table II are the actual wavelengths we assumed in the calculation for the unperturbed chromophores.

Transition monopole charges for transitions between excited states of the phenol group were calculated from Nishimoto and Fujishiro's wave functions.[24] The π-electron dipole moment for phenol was calculated to

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Table II. Characteristics of Electronic Transitions in Phenol

Transition	Wavelength, λ, nm	Polarization[a]	Oscillator strength Calcd[b]	Oscillator strength Obsd[c]	Correction factor[d]	Transition moment,[e] μ, D
T1	277	⊥	0.022	0.020		1.14
T2	227	∥	0.251	0.132	0.716	2.45
T3	192.5	⊥	0.963	0.555[f]	0.751	4.74
T4	192.5	∥	0.951	0.549[f]	0.751	4.68

[a] Polarization direction is defined with respect to the twofold axis of the π-electron system of phenol (the CO bond direction). [b] Calculated from Nishimoto and Fujishiro's wave functions.[24] [c] See ref 26. [d] The factor required to bring theoretical and experimental transition dipole moments into agreement. [e] Experimental and adjusted theoretical values. [f] The total oscillator strength (1.103) was divided between T3 and T4 in proportion to their calculated oscillator strengths.

Table III. Monopole Charges[a] for Phenol Transitions and for Ground-State Permanent Dipole Moment

	Transitions from ground state				Transitions between excited states						Permanent
Center[b]	T1	T2	T3	T4	T1–T2	T1–T3	T1–T4	T2–T3	T2–T4	T3–T4	monopole
C_γ		0.6638		−0.2433		0.6494			0.0620		0.0687
C_{δ_1}	0.0132	−0.3398	−0.4610	−0.5510	−0.2810	−0.4083	0.5243	−0.0709	0.0293	−0.1633	−0.0043
C_{ϵ_1}	−0.2500	0.3997	−0.5160	0.5290	0.2827	−0.3535	−0.6434	−0.1672	−0.0305	0.0730	0.0903
C_ζ		−0.6178		0.2265		0.6102			0.0807		−0.0879
C_{ϵ_2}	0.2500	0.3997	0.5160	0.5290	−0.2827	−0.3535	0.6434	0.1672	−0.0305	−0.0730	0.0903
C_{δ_2}	−0.0132	−0.3398	0.4610	−0.5510	0.2810	−0.4083	−0.5243	0.0709	0.0293	0.1633	−0.0043
O_η		−0.1655		0.0608		0.2632			−0.1407		−0.1537
O											−1.6000
H											1.6000

[a] Units of 10^{-10} esu. [b] Each center in the π-electron system (the carbons and $O\eta$) has two monopoles—one above and one below the plane.[21] The charges given are those for each monopole. O and H are the OH σ-bond monopoles and are located at their respective nuclei.

Table IV. Calculated Rotational Strengths[a] for PLT Decamers in Various Conformations

	Band (λ_{max}, nm)					
Conformation	T1 (277)	T2 (227)	n–π^* (220)	C[b] (200)	B[b] (193)	A[b] (185)
R1	−0.1035	−0.4814	−0.3320	−1.8891	0.6176	2.1885
RA	0.0555	0.2517	−0.2606	3.8492	−4.0440	0.1482
L1	−0.0162	−0.2484	0.4018	1.3981	−1.0075	−0.5277
LA	0.0032	−0.0928	0.1897	−2.6368	1.8120	0.7247
R1′ [c]	−0.1105	−0.3918	−0.3509	−1.7792	0.2230	2.4094
RA′ [c]	0.0537	0.2276	−0.2621	4.1193	−4.0841	−0.0543
L1′ [c]	−0.0099	−0.2682	0.4067	1.6117	−1.7428	0.0024
LA′ [c]	0.0040	−0.1079	0.1929	−2.8490	2.0658	0.6943

[a] Calculated rotational strengths are given in units of DBM = debye bohr magneton = 0.9273×10^{-38} cgs unit. [b] Bands A, B, and C are summed over transitions 1–10, 11–20, and 21–30, respectively. The wavelengths given for these bands are the approximate centers of the bands and may differ by several nanometers from the extremum obtained by summing the corresponding partial CD curves. [c] These conformations differ from the corresponding unprimed ones by rotation of the phenolic ring by 180° about its twofold axis (increasing χ_2 by 180°). This is equivalent to rotating the phenolic OH group by 180°.

be 1.04 D. The side-chain OH bond has a dipole moment of 1.60 D[14] with H positive. The dipole moments of the phenol CH bonds are small compared with other contributions and were neglected. In this approximation, the dipole moment of phenol was calculated to be 1.50 D, which agrees well with the experimental value for phenol of 1.55 D.[29] Thus, the calculated π-electron densities were used for the permanent π-electron monopoles above and below the phenol plane, and the σ-bond moment of the OH group was approximated by appropriate charges at the O and H nuclear positions. The center of the benzene ring was used as the center for the phenolic transitions. The transition monopole charges and permanent monopole charges for the phenol group are summarized in Table III.

In reporting rotational strengths, it is convenient to take groups of N levels, rather than giving all MN rotational strengths. Because the T1, T2, and n–π^* transitions have small or zero oscillator strengths, the splittings within these bands are at most a few nanometers. Clearly strong coupling[19] rules do not apply, and we must sum over the N levels which make up each band. The three fully allowed transitions do show large splitting, and strong coupling rules are plausible. However, a good qualitative picture of the distribution of rotational strengths is obtainable from summing over groups of N levels.

For comparison with experiment, we have calculated CD curves assuming Gaussian partial CD bands[30] associated with each transition. Again, for the three long-wavelength transitions, we have summed over the band and used only the net rotational strength, but for polymer transitions arising predominantly from the electrically allowed transitions, we have used one partial CD curve for each discrete level. An arbitrary value of 10 nm was assumed for the band width[31] for all partial CD bands.

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(29) A. L. McClellan, "Tables of Experimental Dipole Moments," W. H. Freeman, San Francisco, Calif., 1963, p 193.

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(31) The band width is here defined as the difference in wavelength between the CD maximum and the point on either side of the maximum where the partial CD curve falls to 1/*e* of its maximum value, *e* being the base of natural logarithms.

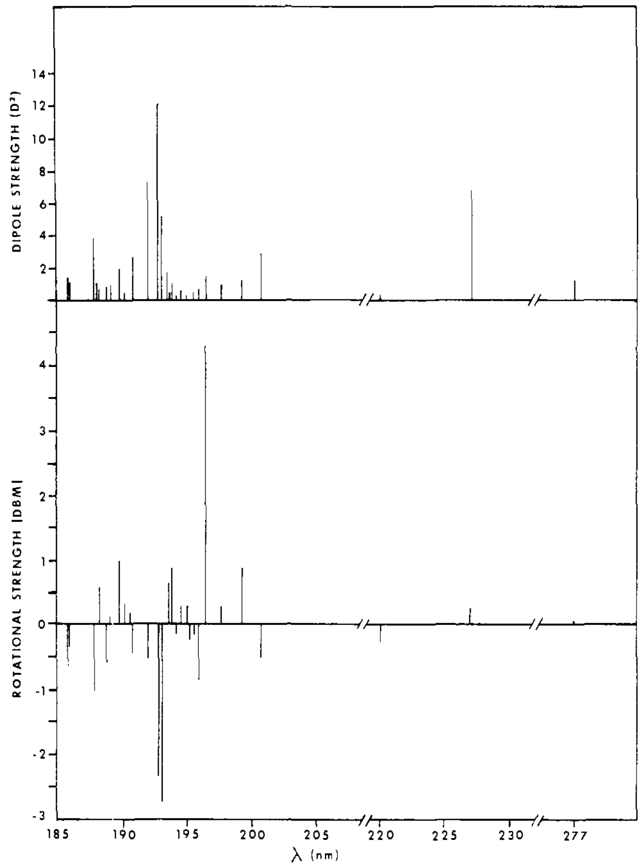

Figure 1. The distribution of dipole (above) and rotational strengths (below) for transitions in a PLT decamer (RA).

Results and Discussion

Calculated rotational strengths for a decamer ($N = 10$) of each of the conformations considered are summarized in Table IV. Figure 1 gives a more detailed picture of the distribution of oscillator and rotational strengths among the various levels for a particular conformation (RA). Figures 2 and 3 show the calculated CD curves for, respectively, the right- and left-handed conformations.

The tyrosyl transition at 277 nm (T1) has a small rotational strength, especially in the left-handed conformations. This transition has a relatively small transition moment and is well separated in energy from the other transitions considered. Calculations in which only the phenolic transitions were considered showed that for the right-handed conformations, most of the rotational strength arises from mixing with the other phenolic transitions, especially the T2 and T4 transitions. In the left-handed conformations, mixing of the T1 transition with the amide π–π^* transition contributes a substantial fraction of the rotational strength.

The rotational strength of the T2 transition is very strongly affected by mixing of phenolic and amide transitions in all of the conformations except R1. In fact, calculations including only the phenolic transitions lead to the wrong sign for the T2 rotational strength in every case except for the R1 conformation. In the case of the RA conformation, which we have analyzed in most detail, the T2 transition couples more strongly with the amide π–π^* transition than with the n–π^* transition by about a factor of 5, in spite of the near degeneracy with the n–π^* transition.

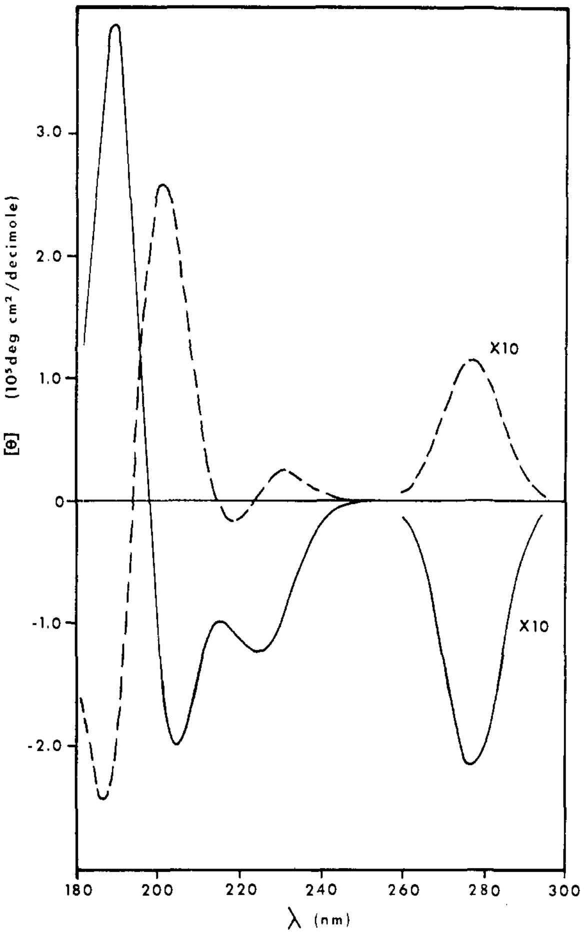

Figure 2. Calculated CD curves for PLT decamers in right-handed α-helical conformations: Rl, ——; RA, -----.

There is no consistent relationship between the rotational strengths for the T1 and T2 transitions. In three of the four cases, the rotational strengths for T1 and T2 have the same sign, but in the fourth they have opposite signs. In all cases, T2 has a substantially larger rotational strength, but the ratio ranges from 5 to 30.

For each conformation, the sign of the n–π^* rotational strength is the same as that in simple helical polypeptides of the same screw sense. Only in the case of the LA conformation does coupling with phenolic transitions decrease the magnitude of the n–π^* rotational strength, and there the coupling is quite weak. Of the phenolic transitions, the n–π^* transition couples most effectively with the T2 transition. Thus, in poly-L-tyrosine, the sign of the n–π^* rotational strength is diagnostic of the helix sense, although there is no guarantee that this will be the case in other systems.

In the far-ultraviolet, below 205 nm, mixing of the peptide π–π^* transition and the phenolic T3 and T4 transitions is very strong. For example, in the RA conformation, the level at 195.9 nm with a rotational strength of −0.86 DBM (Figure 1) is about 40% amide π–π^*, 20% T3, and 40% T4.

Beychok and Fasman[10] and Quadrifoglio and Urry[32] reported CD spectra for PLT in aqueous solution at high pH (11.2 and 10.8, respectively). Alkaline conditions, unfortunately, are necessary to obtain an ap-

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[32] F. Quadrifoglio and D. W. Urry, *Annu. Rev. Phys. Chem.*, **19**, 477 (1968).

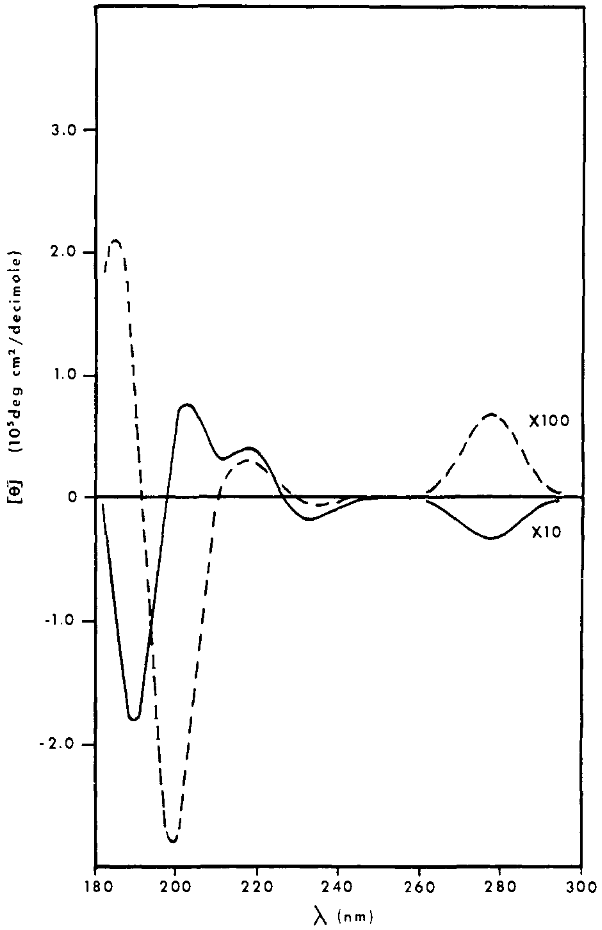

Figure 3. Calculated CD curves for PLT decamers in left-handed α-helical conformations: L1, ——; LA, -----.

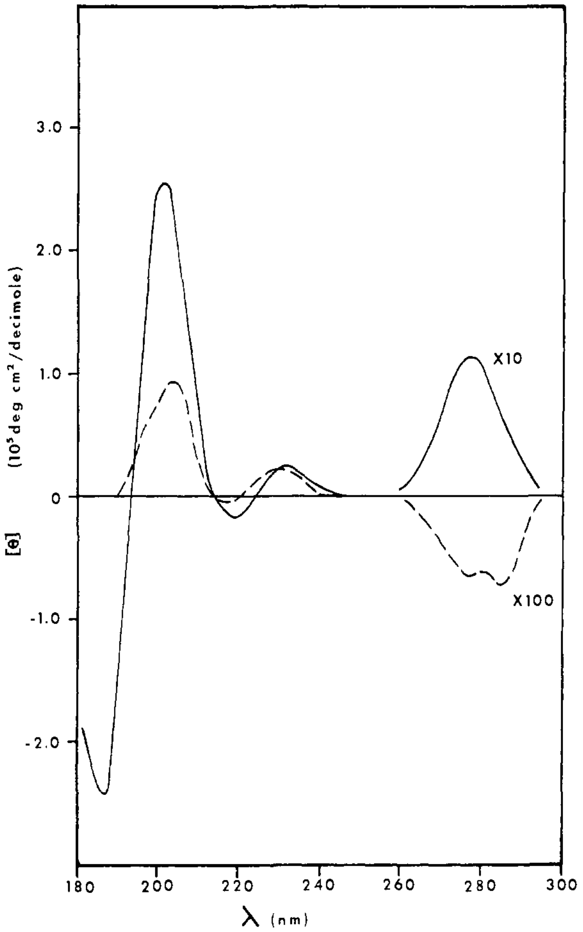

Figure 4. Comparison of calculated (——) CD curve for PLT decamer in the RA conformation with experimental (-----) curve (experimental values taken from M. Shiraki and K. Imahori, *Sci. Pap. Coll. Gen. Educ., Univ. Tokyo*, **16**, 215 (1966).

preciable degree of solubility of PLT in aqueous solutions. Although the absorption spectra of PLT at pH 11.2 reported by Fasman, *et al.*,[9] indicate only a small degree of ionization, the CD spectra at both pH 10.8 and 11.2 indicate that this small degree of ionization has rather profound effects. In particular, both groups[10,32] reported a CD band at about 245 nm. Tyrosine does not show an absorption band at this wavelength at neutral pH, but at high pH, where the phenolic group is ionized, an absorption band appears at about 240 nm.[28] This tyrosinate anion band undoubtedly corresponds to the 225-nm band of neutral tyrosine.

Shiraki and Imahori[33] have studied PLT in methanol. Their data show no CD maximum in the 240-nm region, but a maximum at 230 nm. Therefore, we shall compare our calculated CD curves with the data of Shiraki and Imahori. They reported a very weak negative ($[\theta] \approx -700$) band at about 280, a positive band ($[\theta] \approx 24{,}000$) at 230, a weak negative band at 216 ($[\theta] \approx -4000$), and a strong positive band at 203 nm ($[\theta] \approx 98{,}000$).

Studies in this laboratory[34] of a block copolymer $(\text{D,L-glu})_{50}(\text{L-tyr})_{50}(\text{D,L-glu})_{50}$, soluble in aqueous solution at neutral pH, agree qualitatively with the results of Shiraki and Imahori,[33] except that we find that the 280-nm band has a small positive rotational strength and the trough at 216 nm is somewhat more pronounced.

Consideration of Table IV and of Figure 2 and 3 shows that of the four conformations considered, only the RA conformation has a CD spectrum consistent with the experimental data below 250 nm. For the RA conformation, we predict a positive 227-nm band, a negative 220-nm band, and a positive 200-nm band, in agreement with experiment. Each of the other conformations is predicted to have CD bands in one or more of these regions which disagree in sign with experiment. The CD band at 280 nm is calculated to be positive for the RA conformation, in disagreement with Shiraki and Imahori.[33] However, our data for aqueous solution[34] indicate a positive sign for the 280-nm band. Results to be presented later suggest that this band may be especially sensitive to external perturbations such as solvent effects, and this may well account for the apparent discrepancy.

In Figure 4, we compare the calculated CD curve for a decamer helix in the RA conformation with the experimental curve of Shiraki and Imahori.[33] Qualitatively, the agreement is good. Quantitative agreement cannot be expected because of two factors: (a) the arbitrary choice of a uniform band width of 10 nm, which will affect the amplitude of the individual partial CD bands and the extent of overlap; and (b) mobility of the side chains, predicted (see below) to have a

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[33] M. Shiraki and K. Imahori, *Sci. Pap. Coll. Gen. Educ., Univ. Tokyo*, **16**, 215 (1966).

[34] C. Geary and R. W. Woody, unpublished data.

Table V. Calculated Rotational Strengths[a] for Five-Transition Model for $N = 10\text{–}20$

Conformation (N)	Band (λ_{max}, nm)				
	T2 (227)	n–π^* (220)	C[b] (200)	B[b] (193)	A[b] (185)
R1 (10)	−0.5153	−0.3396	−2.0453	0.7854	2.1148
R1 (20)	−0.7009	−0.4700	−4.6448	5.0712	0.7442
RA (10)	0.2609	−0.2607	3.8267	−3.9313	0.1045
RA (20)	0.2500	−0.2853	3.9667	−4.7357	0.8042
L1 (10)	−0.2420	0.3992	1.3910	−1.0020	−0.5462
L1 (20)	−0.2499	0.5093	2.3883	−4.2592	1.6115
LA (10)	−0.0877	0.1897	−2.6129	1.7906	0.7204
LA (20)	−0.0449	0.2178	−2.7679	2.5275	0.0676

[a] Rotational strengths in DBM (see footnote *a* of Table IV). [b] Bands A, B, and C are summed over transitions $1 \rightarrow N$, $N + 1 \rightarrow 2N$, $2N + 1 \rightarrow 3N$, respectively. See footnote *b* of Table IV.

Table VI. Effect of Chain Length and of Position of Peptide π–π^* Transition on Calculated CD Curves

Conformation	N	$\lambda_{\pi-\pi^*}$, nm	Extrema					
			λ_{max}, nm; $[\theta]_{max}$, 10^3 (deg cm^2)/dmol					
R1	10	196	173; −17	189; 386	204; −201		224; −123.0	277; −21.5
R1	20	196	175; −101	190; 735	203; −402		224; −180.0	
R1	10	190	172; −38	188; 455	202; −264		225; −112.0	277; −21.5
RA	10	196		186; −233	201; 257	218; −16.5	231; 23.7	277; 11.5
RA	20	196		185; −211	200; 265	218; −24.3	231; 22.3	
RA	10	190		185; −180	201; 229	218; −11.9	231; 20.1	277; 11.6
L1	10	196	177; 73	190; −183	203; 76	218; 41.0	233; −17.3	277; −3.4
L1	20	196	177; 126	191; −401	207; 206		234; −14.2	
L1	10	190	175; 117	189; −261	202; 105	217; 38.5	232; −16.7	277; −3.4
LA	10	196		185; 211	199; −278	216; 31.1	233; −5.3	277; 0.7
LA	20	196		185; 252	198; −370	215; 55.7	238; −0.9	
LA	10	190		182; 134	198; −179	219; 17.2	235; −2.1	277; 0.5

significant effect on the magnitude of the 280- and 200-nm bands.

Thus, these calculations on decamers provide strong support for a right-handed helix in PLT in solution, in agreement with previous ORD[7,9] and CD[10] studies and with the conformational energy calculations of Scheraga and coworkers.[14,15] The side-chain conformation, however, differs from that predicted to be most stable by Ooi, *et al.*,[14] and resembles more closely that assumed by Applequist and Mahr[13] for a right-handed PLT helix. Before accepting this conclusion, however, several important questions need to be answered concerning the sensitivity of the calculated results to various factors.

Are end effects serious? Calculations on simple α helices[20] show that all of the qualitative features of the infinite polymer are expressed in a decamer, but we cannot be sure that this holds also for PLT. Therefore, we have also carried out calculations on a 20-mer for each of the four hypothetical conformations.

Were we to consider all six transitions in a 20-mer, we would be confronted with a 120×120 secular determinant, which would consume far too much computer time. The 280-nm band mixes only slightly with other transitions, and therefore a five-transition model was considered, in which the T1 transition was neglected. Calculations were first performed on a decamer, neglecting the T1 transition. The resulting combined rotational strengths are given in Table V. Comparison with the data in Table IV shows that the effect of omitting the T1 transition on the rotational strengths in other regions is small. We then carried out a five-transition model calculation on a 20-mer. The rotational strengths of the n–π^* and T2 transitions differed by at most 10–20% from those for the decamer. Rotational strengths in the far-ultraviolet were substantially larger for the 20-mer compared to the decamer, as is found in simple helical polypeptides.[20] CD curves calculated with these rotational strengths showed extrema which were similar in position to those for decamers (Table VI). For the RA and LA conformations, the amplitudes show only moderate changes on going from the decamer to the 20-mer. For the R1 and L1 conformations, there is a very substantial increase in amplitude of the 20-mer over those of the decamers. Even here, however, the qualitative characteristics of the CD curves do not change with increasing chain length. Thus, end effects, though present in the decamer calculations, cannot affect any conclusions based on the general patterns of the CD curves.

Are the results sensitive to the energy separation of the tyrosyl T3 and T4 transitions and the peptide π–π^* transitions? In the calculations we have described, we have located the peptide π–π^* transition at 196 nm, rather than 186–188 nm found by Nielsen and Schellman[35] for the wavelength of the π–π^* transition in secondary amides. The choice of 196 nm was found by Woody[21] to give good agreement with the observed exciton components in simple helical polypeptides. Shifts of several nanometers in the center of gravity of an allowed transition can occur in a crystal or a polymer owing to differences in the interaction of excited-state and ground-state dipole moments with the permanent field of the surroundings.[36] In calculations on PLT, one might worry that the positioning of the amide π–π^* transition relative to the T3 and T4 transitions may be crucial for the CD pattern in the far-ultraviolet. If one locates the T3 and T4 transitions at 192.5 nm, as we have done here, the ordering of peptide and phenolic far-uv transitions is reversed, depending on whether the peptide π–π^* transition is located at 190 or 196 nm.

Therefore, calculations have been performed for a decamer exactly as described above, except that the peptide π–π^* transition is located at 190 rather than 196 nm. CD curves calculated from the resulting ro-

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(35) E. B. Nielsen and J. A. Schellman, *J. Phys. Chem.*, 71, 2297 (1967).

(36) D. S. McClure, *Solid State Phys.*, **8**, 1 (1959).

Table VII. Calculated CD Extrema for Variants[a] of the RA Conformation

Conformation	χ_1, deg	χ_2, deg	Extrema: λ_{max}, nm; $[\theta]_{max}$, 10^3 (deg cm^2)/dmol				
RA_{II}	180	280	187; −204	201; 221	217; −16.5	230; 27.1	277; 7.0
RA_1	190	280	187; −124	201; 136	217; −16.3	230; 27.3	277; 2.9
RA_2	190	290	190; −43	203; 47	217; −15.6	230; 24.3	277; 0.003
RA_3	180	290	188; −118	202; 132	217; −16.0	231; 23.3	277; 3.6
RA_4	170	290	187; −207	202; 228	218; −17.1	231; 22.1	277; 9.2
RA_5	170	280	187; −257	201; 278	218; −15.5	231; 27.2	277; 11.5
RA_6	170	270	186; −214	201; 236	218; −13.1	231; 28.3	277; 10.9
RA_7	180	270	186; −232	201; 252	218; −17.1	230; 28.2	277; 8.6
RA_8	190	270	187; −189	201; 207	217; −17.9	230; 28.4	277; 5.5

[a] Backbone conformational angles are $\varphi = 132°$, $\psi = 123°$.

tational strengths show only quantitative differences from those previously described. The location and amplitude of extrema are given in Table VI. This result is not as surprising as it might seem at first sight. Consider the first-order perturbation theory expression for the rotational strength arising from the coupling of two electrically allowed transitions[19]

$$R_{i\alpha} = -\frac{2\pi}{c}\frac{V_{i0\alpha;j0\beta}\nu_\alpha\nu_\beta\mathbf{R}_{ij}\cdot(\boldsymbol{\mu}_{j0\beta} \times \boldsymbol{\mu}_{i0\alpha})}{h(\nu_\beta^2 - \nu_\alpha^2)} \qquad (6)$$

$$R_{j\beta} = -\frac{2\pi}{c}\frac{V_{i0\alpha;j0\beta}\nu_\alpha\nu_\beta\mathbf{R}_{ji}\cdot(\boldsymbol{\mu}_{i0\alpha} \times \boldsymbol{\mu}_{j0\beta})}{h(\nu_\alpha^2 - \nu_\beta^2)} \qquad (7)$$

Suppose that $R_{i\alpha}$ is positive and $R_{j\beta}$ is therefore negative and that $\nu_\alpha < \nu_\beta$. Then the resulting CD curve will be positive on the long-wavelength side and negative on the short-wavelength side. If ν_α and ν_β are interchanged, the $R_{i\alpha}$ and $R_{j\beta}$ will each change sign, but the resulting CD curve will be unchanged.

Does the orientation of the phenolic OH group affact the results? As mentioned above, the effect of rotating the phenolic OH by 180° is equivalent to changing χ_2 by 180°. This was found to have only a small effect on the conformational energy by Scheraga and coworkers.[14,15] Therefore, there is a possible ambiguity in the angle χ_2 of 180°. We carried out calculations on conformations obtained by changing χ_2 by 180° for each of the four conformations, designating these new conformations as R1′, RA′, L1′, and LA′. The resulting combined rotational strengths are given in Table IV. It can be seen that the results are not sensitive to the orientation of the phenolic -OH.

Are the results sensitive to small variations in the side-chain conformational angles, χ_1 and χ_2? If our results are highly sensitive to the exact values of χ_1 and χ_2, their qualitative agreement with experiment for a particular conformation may well be fortuitous. Furthermore, some flexibility in the side-chain orientation is highly probable (see below). Therefore, we have considered variants of three (R1, RA, LA) of the four conformations considered. The backbone angles φ and ψ used were those chosen by Ooi, *et al.*,[14] for constructing the side-chain conformation energy contours shown in Figures 15 and 16 of ref 14. These differ slightly from the φ and ψ angles corresponding to the minimum energy conformations. χ_1 and χ_2 values approximately corresponding to the energy minima in Figures 15 and 16 of ref 14 were then varied by ±10°. Thus we examined conformations at corners of a square 20° on a side and centered at the various minima. The angles φ, ψ, χ_1, and χ_2 considered are given in Table VII for the RA conformation. Rotational strengths for decamers having these conformations were calculated and CD curves generated as described above. The positions and amplitudes of the CD extrema are shown in Table VII. Comparison of these results among themselves and with the data in Table VI shows that variations of ±10° in the side-chain conformational angles do not change the qualitative characteristics of the CD curves for the RA conformation. The amplitudes of the 230-nm band (T2) and the 220-nm band (n−π^*) are quite insensitive to these variations. The amplitudes of the 277-nm band (T1) and the 200-nm band, however, show larger variations. Similar results were obtained for the R1 and LA conformations.

The difference in behavior of the T2 and T1 transitions can be understood by considering the difference in polarization directions for these two transitions. The T2 transition is polarized parallel to the two-fold axis of the phenolic ring and therefore its transition moment direction is not affected by the angle χ_2. By contrast, T1 is polarized perpendicular to the axis and changes in χ_2 alter its direction.

One problem which we have not attempted to deal with is that of disorder in the side chains. We have assumed that the side chains all have the same conformational angles and therefore form a regular helical array. This surely is not true in an exact sense, if only because of Brownian motion. However, we believe that our demonstration that small variations in regular conformations do not seriously perturb the calculated CD pattern suggests that a small degree of disorder will also have only small effects.

Thus, we have shown that our results are insensitive, at least qualitatively, to a number of factors which might lead to ambiguities in comparison with experiment. Therefore, the agreement of the calculated CD curve for the RA conformation with the experimental CD curve leads us to assign the RA conformation to PLT. While agreeing with Scheraga and coworkers[14,15] on the helix sense, our conclusion about the side-chain conformation appears, at first sight, in conflict with their identification of the R1 conformation as having minimum potential energy. However, examination of the side-chain conformation energy contours given in Figures 15 and 16 of ref 14 suggests an explanation. The energies which Scheraga and coworkers calculated were *potential energies*, not free energies. The potential minimum at the R1 conformation is about 1 kcal *deeper* than that at the RA conformation, but the potential well at the RA conformation is much *broader* than that at the R1 conformation. This latter effect implies that the *entropy* of the RA conformation is substantially more positive than that of the R1 conformation. We therefore suggest that the RA conformation is actually the most favorable conformation in terms of free energy. A range of side-chain conformations about the RA conformation therefore appears probable. We have previously seen that the qualitative features of the calculated CD curve are not highly sensitive to modest (± 10°) variations in χ_1 and χ_2. However, the amplitude of the 280-nm band does show larger variations, and, in fact, the band nearly vanished for one of the variants of the RA conformation considered (Table VII). This may account for the difference in sign of the 280-nm band observed by Shiraki and Imahori[33] (in methanol) and in our laboratory[34] (aqueous solution). Solvent effects could modify the distribution of side-chain conformations and thereby change the magnitude and perhaps even the sign of the 280-nm band.

There appears to be no way in which we can reconcile our calculations with the results of Applequist and Mahr.[13] Goodman, *et al.*,[4] suggested that PLT in quinoline (the solvent used by Applequist and Mahr for their dipole moment measurements) may indeed form left-handed helices, while forming right-handed helices in the more conventional solvents used for optical rotatory studies. However, this appears unlikely in view of the observation by Applequist and Mahr that the visible ORD behavior of PLT in quinoline is very similar to that in other solvent systems.

We can only conclude that a key assumption in Applequist and Mahr's argument may well be incorrect. They assumed that the conformation of PLT and of its bromo derivative are essentially the same and attributed all of the change in dipole moment to the change in the dipole moment of the side-chain phenol upon bromination. Goodman, *et al.*,[4] have summarized results from several laboratories which show that introducing para substituents such as nitro, methyl, chloro, and cyano groups in poly-β-benzyl-L-aspartate causes a reversal in the sense of the helix. It is therefore plausible that introducing a bromine in the phenolic ring of poly-L-tyrosine may at least cause a significant change in side-chain conformation.

Summary

In this study, we have examined theoretically the circular dichroism of four helical conformations of poly-L-tyrosine—two right-handed and two left-handed helices. We have shown that the qualitative features of the calculated CD curves are insensitive to chain length, to relative energy of the allowed $\pi-\pi^*$ transitions in the backbone and side chains, and to small variations in side-chain conformational angles. Of the four considered, only the RA conformation yields even qualitative agreement with experiment, and, for this conformation, the agreement is strikingly good. This investigation provides strong evidence, therefore, that poly-L-tyrosine does form right-handed helices in solution. It also indicates that the favored side-chain conformation is not that of lowest potential energy (R1), but one with a broader potential well and presumably more positive entropy. This ability to assign both the helix sense and side-chain conformation provides further evidence of the potential usefulness of combining conformational energy calculations with theoretical and experimental optical rotatory studies.[37-39]

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