Biophysical Chemistry, 38 (1990) 225–230
Elsevier

BIOCHE 01511

Influence of the solvent on the conformational-dependent properties of random coil polypeptides

II. Mean square optical anisotropies and Kerr constants

Guillermo Rowe and A. López Piñeiro

Departamento de Química Física, Facultad de Ciencias, Universidad de Extremadura, Badajoz E-06071, Spain

Received 12 December 1989
Revised manuscript received 8 May 1990
Accepted 17 May 1990

Conformational properties in solution; Mean squared optical anisotropy; Molar Kerr constant; Optical properties of polypeptides

Mean square optical anisotropies and molar Kerr constants were calculated for homopolypeptides of the 20 natural amino acids and of several enzymes and proteins in the random-coil state. The effect of hydration was taken into account in constructing the molecular potential that gives the conformational energies as a function of the rotational angles ϕ and ψ of the backbone and χ^1 of the side chain. The Rotational Isomeric State model was used in calculated energies, the Valence Optical Scheme and the matrix calculus technique of Flory being employed in the evaluation of the optical properties. The results are compared with calculations for the same substances that were performed without taking into account the solvent, as well as with other similar studies. The Kerr constant is confirmed as being one of the most sensitive properties of a given polypeptide to the residue class and to the sequence of those residues.

1. Introduction

In our ongoing study of polypeptides [1–5], we have modified the potential function that represents the conformational energies of these substances to include the effect of the solvent, in our case water, defined in terms of a modified hydration shell model [6]. We first calculated the conformational energies of the *N*-acetyl-*N'*-methylamides of the 20 natural hydrated amino acids as functions of the rotational angles of the backbone (ϕ, ψ) and of the side chain (χ^1) as shown in fig. 1.

We used this set of energies to calculate diverse conformational properties of interest in the study and characterization of polymers. These magnitudes are the dimensions $\langle r^2 \rangle$ and dipole moments $\langle \mu^2 \rangle$ presented in a previous publication, [5] and two optical properties: the mean square optical anisotropy $\langle \gamma^2 \rangle$ and the molar Kerr con-

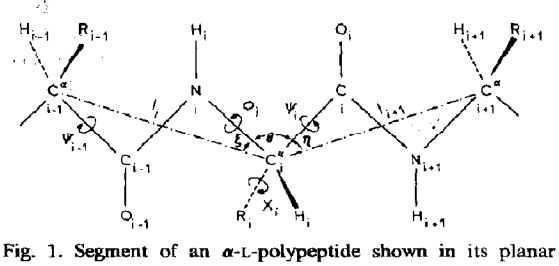

Fig. 1. Segment of an α-L-polypeptide shown in its planar all-*trans* conformation. Virtual bonds connecting consecutive α-carbons are shown as dashed lines.

Correspondence address: A. López Piñeiro Departamento de Química Física, Facultad de Ciencias, Universidad de Extremadura, Badajoz E-06071, Spain.

stant $\langle {}_{m}K \rangle$ of homopolypeptides and of various enzymes and proteins with a known sequence.

These last properties were calculated following the same procedure in obtaining $\langle r^2 \rangle$ and $\langle \mu^2 \rangle$, i.e., applying the Rotational Isomeric State model [7] (RIS) and the matrix calculus technique developed by Flory [8,9], once the energies had been obtained and the average contribution of each amino acid residue to the total magnitude of the polypeptide chain had been evaluated.

The mean square anisotropy $\langle \gamma^2 \rangle$, a magnitude directly related to the scattering of light by the particles [10], can be determined if we know the tensor $\hat{\alpha}$, the anisotropic part of the polarizability tensor of each repetitive unit that intervenes in the polymer under study. We calculated these by summing bond contributions using the Valence Optical Scheme [11] (VOS) according to which these contributions are characteristic of each bond and independent of the conformation of the molecule and of the chemical environment in which the bond finds itself.

For the determination of the molar Kerr constant $\langle {}_{m}K \rangle$, which characterizes the electro-optical birefringence [12], one needs to know not only the anisotropic part of each unit's polarizability tensor but also its corresponding dipole moment. These values have been calculated previously in both hydrated and non-hydrated peptides [3,5].

In section 2, we briefly describe the form of calculating the optical anisotropy and the Kerr constant of polypeptides and, in section 3, discuss the influence of hydration on these two properties.

2. Method of calculation

It is well known that polypeptides present quite special structural characteristics that notably simplify their conformational treatment. We have used the geometrical parameters proposed by Scheraga and co-workers [13,14] for the bond lengths and angles.

In the conformational study of hydrated polypeptides, we have incorporated certain polymer-solvent interactions into the potential energy, taking the models proposed by Hopfinger [15] and Scheraga [6] as reference. The expression for $E(\phi,\psi,\chi^1)$ that we derive has been described elsewhere [5].

2.1. Optical anisotropy

The square of the optical anisotropy of a given molecule is given by [10]

$$\gamma^2 = \tfrac{3}{2}\mathrm{Tr}(\hat{\alpha}\hat{\alpha}) \tag{1}$$

where Tr is the trace and $\hat{\alpha}$ the anisotropic part of the polarizability tensor of the molecule.

For a polymer chain, using the VOS together with the matrix calculus technique [8,9], with all the transformations therein implied, one is left with

$$\gamma^2 = \frac{3}{2} \prod_{i=1}^{x} \boldsymbol{P}_i \tag{2}$$

where $\boldsymbol{P}_i$ is an 11×11 matrix defined for unit i in the form [8,9]

$$\boldsymbol{P}_i = \begin{bmatrix} 1 & 2\hat{\alpha}^{\mathrm{R}}\,(T \oplus T) & \hat{\alpha}^2 \\ 0 & (T \oplus T) & \hat{\alpha}^{\mathrm{C}} \\ 0 & 0 & 1 \end{bmatrix}_i \tag{3}$$

Terms P_1 and P_x of eq. 2 represent the first row and last column, respectively, of the matrices $\boldsymbol{P}_i$ defined by eq. 3. The elements $\hat{\alpha}_i^{\mathrm{R}}$ and $\hat{\alpha}_i^{\mathrm{C}}$ represent a row vector and a column vector which contain the nine elements of $\hat{\alpha}_i$ set out in natural reading order left-to-right and row-by-row. The symbol $\oplus$ represent the matrix direct product.

The tensors $\hat{\alpha}_i$ for the repetitive units were calculated by summing the polarizabilities of bonds or groups of bonds of their component atoms, using values duly contrasted in similar studies [16,17] and, in those cases where no data exist, using values of previously optimized model compounds reproducing experimental results of γ^2 and ${}_{m}K$ for various amides [3].

As we are interested in calculating mean magnitudes for comparison with experimental measurements, the mean-square optical anisotropy $\langle \gamma^2 \rangle$ can be determined by applying eq. 2 to all the conformations available to the polymer, substituting the elements of the matrix $\boldsymbol{P}_i$ by their corresponding averages over the rotational angles ϕ, ψ, and χ^1, except for the cases of glycine and

proline that are treated differently due to their special structural characteristics. Eq. 2 could therefore be rewritten as

$$\langle \gamma^2 \rangle = \frac{3}{2} \prod_{i=1}^{x} \langle \boldsymbol{P}_i \rangle. \tag{4}$$

2.2. Kerr constant

The molar Kerr constant which is related to the electrical birefringence that results when certain substances, under specific conditions, are submitted to the action of an external electric field, is defined as [12]:

$${}_{\mathrm{m}}K = \frac{2\pi N_{\mathrm{A}}}{15kT}\left[\boldsymbol{\mu}^T \hat{\boldsymbol{\alpha}} \boldsymbol{\mu} (kT)^{-1} + \mathrm{Tr}(\hat{\alpha}\hat{\alpha}')\right] \tag{5}$$

where N_{A} is Avogadro's number, k Boltzmann's constant, $\boldsymbol{\mu}$ the dipole moment and $\boldsymbol{\mu}^T$ its transpose, $\hat{\alpha}$ the anisotropic part of the polarizability tensor, and $\hat{\alpha}'$ the same magnitude but extrapolated to a static field.

It has been shown [18] that, as the polypeptides have an appreciable dipole moment, one can neglect in practice the second term of eq. 5 in comparison with the first, leading to the expression

$${}_{\mathrm{m}}K = \frac{2\pi N_{\mathrm{A}}}{15(kT)^2}\left[\boldsymbol{\mu}^T \hat{\boldsymbol{\alpha}} \boldsymbol{\mu}\right]. \tag{6}$$

According to this equation, it is sufficient to evaluate the product $\boldsymbol{\mu}^T \hat{\boldsymbol{\alpha}} \boldsymbol{\mu}$ to calculate the Kerr constant. Applying the VOS and the matrix calculus technique and assuming that $\hat{\alpha}_i$ and $\boldsymbol{\mu}_i$ can be calculated as sums of contributions from each repetitive unit (see refs 3–5), the product $\boldsymbol{\mu}^T \hat{\boldsymbol{\alpha}} \boldsymbol{\mu}$ can be expressed in the form

$$\boldsymbol{\mu}^T \hat{\boldsymbol{\alpha}} \boldsymbol{\mu} = \prod_{i=1}^{x} \boldsymbol{Q}_i \tag{7}$$

where $\boldsymbol{Q}_i$ represents a 26×26 matrix defined as [9]

$$\boldsymbol{Q}_i = \begin{bmatrix} 1 & 2\mu^T T & \hat{\alpha}^{\mathrm{R}}(T \oplus T) & (\mu^T \oplus \mu^T)(T \oplus T) & 2\hat{\alpha}^{\mathrm{R}}(\mu \oplus T) & \hat{\alpha}^{\mathrm{R}}(\mu \oplus \mu) \\ 0 & \mathrm{T} & 0 & (E_3 \oplus \mu^T)(T \oplus T) & \hat{\alpha} T & (E_3 \oplus \mu^T)\hat{\alpha}^{\mathrm{C}} \\ 0 & 0 & T \oplus T & 0 & 2(\mu \oplus T) & \mu \oplus \mu \\ 0 & 0 & 0 & T \oplus T & 0 & \hat{\alpha}^{\mathrm{C}} \\ 0 & 0 & 0 & 0 & T & \mu \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}_i \tag{8}$$

The elements Q_1 and Q_x in eq. 7 represent the first row and last column, respectively, of matrices defined as in eq. 8.

As in the case of γ^2, the elements of $\boldsymbol{Q}_i$ are averaged over the polymer conformations obtained as a function of the rotational angles ϕ, ψ and χ^1. In this way eq. 6 becomes

$${}_{\mathrm{m}}K = \frac{2\pi N_{\mathrm{A}}}{15(kT)^2} \prod_{i=1}^{x} \langle Q_i \rangle. \tag{9}$$

3. Results and discussion

Using the conformational energies that we evaluated recently [5], and in which the potential energy function is modified to incorporate the solvent, we have calculated the mean magnitudes that appear in eqs 3 and 8 to determine the means of the optical anisotropy and the Kerr constant of polypeptides using Flory's matrix calculus technique. The improvements introduced into the potential function with the incorporation of the solvent become manifest on calculation of the end-to-end distance and the dipole moment of polypeptides discussed in a previous publication [5]. We therefore believe the results for the optical properties, discussed below, provide a more realistic approximation to the behaviour of these biomolecules with respect to the magnitudes dealt with in the present work.

3.1. Homopolypeptides

The results for the variation in optical anisotropy $G_x = \langle \gamma^2 \rangle / x$ with degree of polymerization x show two distinct patterns of behaviour depending on the amino acid that constitutes the

repetitive unit. Fig. 2 illustrates the general pattern of behaviour for polymers of Leu, Tyr, Asp, Glu, Lys, Arg, Cys, Phe, Asn, Gln, His, and Trp. Such behaviour is characterized by a gradual increase in G_x with x until a constant value is reached at $x = 50$. The other polymers (Ser, Gly, Ala, Val, Ile, Thr, Met, and Pro) show a fall in G_x as the degree of polymerization increases. These two patterns of behaviour were discussed in depth when the calculation of these magnitudes was studied for the non-hydrated species [3,4]. One observes identical behaviour for the hydrated and non-hydrated species in fig. 2, although they have different asymptotic limits.

Plotting G_x vs $1/x$ and extrapolating the linear part, one obtains G_∞ whose values for the 20 homopolypeptides are given in table 1. One can see that the solvent has the effect of reducing this magnitude in most cases, although the reverse effect is found in some residues, such as Asp, Arg, Lys, Tyr, Pro and Trp. Such variation in the G_∞ values between the hydrated and nonhydrated polypeptides may be explained solely by the appearance of new minimum energy conformations in the hydrated species [19], given the other effects

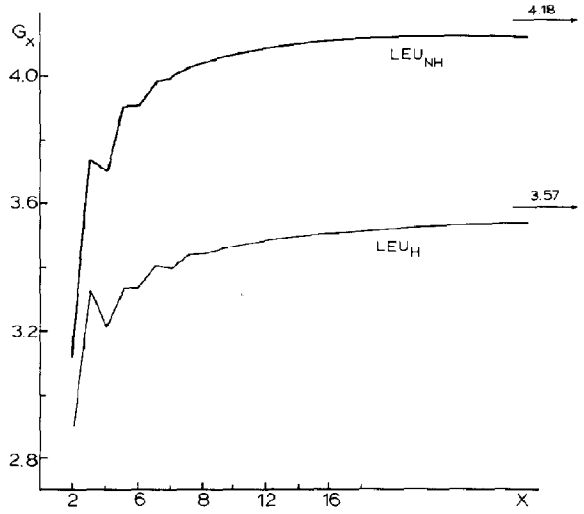

Fig. 2. Variation of G_x (in 10^{-6} nm^6) vs x for the leucine polypeptide: hydrated form $(LEU)_H$ and non-hydrated form $(LEU)_{NH}$.

Table 1

Calculated values for G_∞ (expressed in units of 10^{-6} nm^6) and K_∞ (in units of 10^{-27} V^{-2} m^5 mol^{-1}) for homopolypeptides of the 20 natural α-amino acids

Residue	Non-hydrated [a]			Hydrated		
	G'_∞	G_∞	K_∞	G'_∞	G_∞	K_∞
Glycine	0.81	3.21	253	0.66	2.62	946
Alanine	0.97	3.58	−324	0.73	2.48	145
Valine	1.09	3.84	2539	0.76	2.64	879
Isoleucine	0.46	2.95	−55	0.49	3.05	3117
Leucine	0.99	4.25	175	0.88	3.56	935
Serine	0.58	2.47	796	0.49	2.02	401
Threonine	0.71	4.16	1033	0.60	3.52	1161
Aspartic acid	1.78	15.90	−198	1.93	18.80	−835
Glutamic acid	1.14	8.31	−993	1.23	6.84	690
Lysine	0.79	4.41	413	0.90	4.77	1392
Arginine	0.87	6.53	−1912	1.59	11.57	−22208
Cysteine	0.90	4.00	−3262	0.83	3.07	345
Methionine	0.76	3.74	−873	0.63	2.99	−323
Phenylalanine	0.87	20.90	468	0.56	13.76	−724
Asparagine	1.90	15.84	5759	1.21	10.22	10987
Glutamine	1.37	8.37	−6243	1.05	5.54	2338
Tyrosine	0.77	24.68	−2308	0.83	25.75	−4675
Histidine	0.87	30.08	4664	0.78	27.77	5844
Tryptophan	1.13	97.00	649	1.25	110.90	2894
Proline	0.34	2.84	−331	0.37	3.05	−331

[a] From López Piñeiro et al. [3,4]; $G'_\infty = G_\infty / \langle \gamma^2 \rangle_1$.

such as the influence of the environment on the $\hat{\alpha}$ tensor have been ignored.

The only available data with which we can compare our results are those of Flory and Ingwall [20] for $x = 25$. These results are listed in table 2, together with those obtained by López Pineiro et al. [3] in the absence of solvent. Our results are rather satisfactory in that not only do they reproduce the trend in behaviour, but also an accepta-

Table 2

Comparison of results on G_∞ (in 10^{-6} nm^6) with literature values

Peptide	Other authors [a]	Non-hydrated [b]	Hydrated [c]
Polyalanine	1.70	3.27	2.64
Polyglycine	1.30	3.33	2.50

[a] Ingwall et al. [20].
[b] López Piñeiro et al. [3].
[c] Present work.

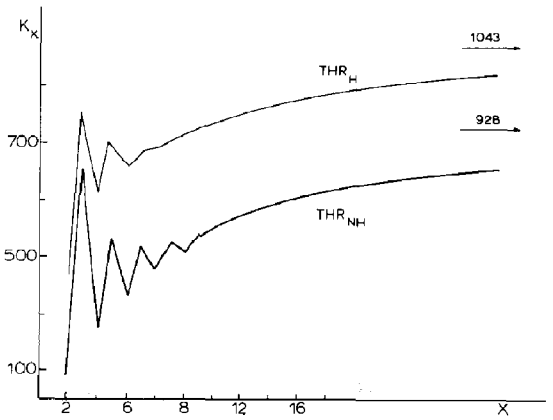

Fig. 3. Variation of K_x (in 10^{-27} V^{-2} m^5 mol^{-1}) vs x for the threonine polypeptide: hydrated form $(THR)_H$ and non-hydrated form $(THR)_{NH}$.

ble value is determined for this magnitude which includes the effect of solvent.

The results for the Kerr constant, $K_x = \langle {}_mK \rangle / x$, are listed in table 1 and depicted graphically in fig. 3. Fig. 3 shows the most general behaviour consisting of sharp oscillations for low molecular weight polymers proceeding from a value of $x \simeq 8$, progressively increasing until the behaviour stabilizes at intermediate degrees of polymerization. The results when extrapolated to infinity in the same form as G_∞ are summarized in table 1. It is observed that, in general, the values for K_∞ in the hydrated species are higher than those for the non-hydrated species. Nevertheless, for some species specific comment is merited. Thus, in the polymers of Val, Cys, and Gln a significant decline in K_∞ is evident on including the solvent, since the values of D_∞ and G_∞ are significantly lower in the hydrated than in non-hydrated species. In general, the behaviour observed for K_∞ reflects the tendencies observed in the dipole moment and optical anisotropy. It is precisely the wide range of values spanned by this magnitude that makes it particularly useful in studies of the conformational characteristics of such systems.

As regards a comparison of our results with those of others, we are aware of only one qualitative review by Yoshioka [21] on polymers of Lys, Asp, and Glu relative to the solvent. With respect to Lys [22], the Kerr constant is reported to increase on addition of water to the polymer in the random-coil state. We find variations from values of 371 without hydration to 1250 with hydration (in units of 10^{-12} $statV.^{-2}$ cm^5 mol^{-1}). Yamoaka [23] reported a lower value for Asp than Glu in a solvent medium, which agrees with our predictions for aqueous solution. Our conclusions are also consistent with ref. 21 in that there is significant influence of the solvent on the property ${}_mK$.

3.2. Heteropolypeptides

The ten proteins and three enzymes studied here yielded the results for the optical anisotropy relationship G_x listed in table 3, which also includes the values calculated for the same compounds in the absence of hydration by López Piñeiro et al. [3]. The small differences in anisotropy between distinct amino acid residues result in small differences between the G_x values of those biomolecules. The 13 compounds present an almost constant G_x (6.07×10^{-6} $nm^6 \leq 8.97 \times 10^{-6}$ nm^6) and the differences that do exist are due to the presence in greater or lesser amounts of highly anisotropic peptide units. In general, we

Table 3

Values of G_x (in 10^{-6} nm^6) for several enzymes and proteins

Peptide	G_x		Repeat units (x)
	Non-hydrated [a]	Hydrated [b]	
Myoglobin	8.20	7.59	152
Lysozyme	9.20	8.97	130
Chymotrypsinogen	7.30	6.96	246
Ribonuclease	6.60	6.07	125
Human hemoglobin α	7.80	7.04	142
Human hemoglobin β	8.00	7.33	147
Human hemoglobin γ	8.20	7.54	147
Human hemoglobin δ	8.00	7.36	148
Horse hemoglobin α	7.70	7.11	142
Horse hemoglobin β	8.30	7.50	147
Bradykinin	6.80	7.13	9
Bovine oxytocin	6.00	6.17	9
Bovine vasopressin	7.40	7.53	9

[a] From López Piñeiro et al. [3].
[b] Present work.

Table 4

Values of K_x (in 10^{-27} V^{-2} m^5 mol^{-1}) for several enzymes and proteins

Peptide	K_x		Repeat units (x)
	Non-hydrated [a]	Hydrated [b]	
Myoglobin	−65	597	152
Lysozyme	−341	−26	130
Chymotrypsinogen	−155	281	246
Ribonuclease	39	263	125
Human hemoglobin α	355	489	142
Human hemoglobin β	14	317	147
Human hemoglobin γ	−76	386	147
Human hemoglobin δ	−278	181	148
Horse hemoglobin α	432	622	142
Horse hemoglobin β	−4	370	147
Bradykinin	629	604	9
Bovine oxytocin	−316	562	9
Bovine vasopressin	72	343	9

[a] From López Piñeiro et al. [4].
[b] Present work.

observe a drop in the value of G_x when the effect of the solvent is included. We have only found one report [24] in the literature on this influence of solvent on the optical anisotropy in polymers, which, while different in nature to our studies, reaches identical conclusions with respect to the dependence of this magnitude on the molecular environment in which it is immersed.

Finally, the results for the Kerr constant of heteropolypeptides of known sequence are given in table 4, together with the values for the same species when non-hydrated [4]. As indicated in section 3.1 on homopolypeptides, the Kerr constant depends on μ^2 and $\hat{\alpha}$ so that the greater the values of D_x and G_x, the larger will ${}_mK$ be, the effect being more notable in the dipole moment than in the anisotropy, due to the greater absolute value of the dipole moment and its being squared in eq. 6. An analysis of the results in table 4 shows the influence of the sequence and of the monomer type on the absolute value of the Kerr constant, due to the different values of each unit. The Kerr constant is also confirmed [25] as the property that is most sensitive to the conformational characteristics of the four properties studied. The general effect of hydration is a rise in the Kerr constant significant enough for its differences to be appreciated from the experimental point of view.

References

1 A. López Piñeiro, F. Mendicuti and E. Saiz, An. Quim. 77 (1981) 476.
2 A. López Piñeiro and E. Saiz, Int. J. Biol. Macromol. 5 (1983) 37.
3 A. López Piñeiro, M.P. Tarazona and E. Saiz, J. Chim. Phys. 80 (1983) 529.
4 A. López Piñeiro, M.P. Tarazona and E. Saiz, Polym. Bull. 10 (1983) 373.
5 G. Rowe and A. López Piñeiro, Biophys. Chem. 36 (1990) 57.
6 Z.I. Hodes, G. Nemethy and H.A. Scheraga, Biopolymers 18 (1979) 1565.
7 T.M. Birshtein and O.B. Ptitsyn, Conformations of macromolecules (Interscience, New York, 1966).
8 P.J. Flory, Statistical mechanics of chain molecules (Interscience, New York, 1969).
9 P.J. Flory, Macromolecules 7 (1974) 381.
10 M.C. Van de Hulst, Light scattering by small particles (Wiley, New York, 1957).
11 M.V. Volkenstein, Configurational statistics of polymeric chains (Interscience, New York, 1963).
12 U.W. Sutter and P.J. Flory, J. Chem. Soc. Farad. Trans. II 73 (1977) 1521.
13 F.A. Momany, R.F. McGuire, A.W. Burgess and H.A. Scheraga, J. Phys. Chem. 79 (1975) 2361.
14 G. Nemethy, M.S. Pottle and H.A. Scheraga, J. Phys. Chem. 87 (1983) 1883.
15 A.J. Hopfinger, Conformational properties of macromolecules (Academic Press, New York, 1973).
16 G.D. Patterson and P.J. Flory, J. Chem. Soc. Farad. Trans. II 68 (1972) 1092.
17 S.P. Liebman and J.W. Moskowitz, J. Chem. Phys. 54 (1971) 3622.
18 G.D. Patterson and P.J. Flory, J. Chem. Soc. Farad. Trans. II 68 (1972) 1111.
19 G. Rowe, Ph.D. Thesis, Universidad de Extremadura (1988).
20 R.T. Ingwall, E.A. Czurilo and P.J. Flory, Biopolymers 12 (1973) 1123.
21 K. Yoshioka, Molecular electro-optics. Part 2. Applications to biopolymers (Dekker, New York, 1978).
22 K. Kikuchi and K. Yoshioka, Biopolymers 12 (1973) 435.
23 K. Yamoaka, Ph.D. Thesis, University of California, Berkeley (1964).
24 B.M. Ladanyi, J. Chem. Phys. 76 (1982) 4303.
25 R.L. Jernigan and S. Miyazawa, Molecular electro-optics (Plenum, New York, 1981) p. 163.