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Kerr Constants of Random Coil Polypeptides

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S U M M A R Y

Calculations of average molar Kerr constants $<_mK>$ of polypeptides of the twenty natural α-aminoacids in the random coil state are presented. The computation was carried out according to the Rotational Isomeric States model with the values of energies, dipole moments and optical anisotropies of the repeating units reported elsewhere. In the case of homopolypeptides, the ratios $<_mK>/x$ extrapolated to $x \to \infty$ range approximately from -6000 to +5000 in units of $10^{-27}V^{-2}m^5mol^{-1}$. Results obtained for ten actual proteins and three enzymes in the random coil state are also reported; their values of $<_mK>/x$ are very sensitive to the kind of aminoacid residues and to the sequence of those residues.

I N T R O D U C T I O N

During the last years we have been carrying out a general study on conformational properties of polypeptides. Thus, we have reported some results of conformational energies and unperturbed dimensions $<r^2>$ (LOPEZ-PIÑEIRO et al. 1981), dipole moments $<\mu^2>$(LOPEZ-PIÑEIRO and SAIZ 1983), and optical anisotropies $<\gamma^2>$ (LOPEZ-PIÑEIRO et al. 1983) of this kind of polymers. As a continuation of that study, the present paper reports some results of molar Kerr constants $<_mK>$ of polypeptides of the twenty natural α-aminoacids calculated for the random coil state.

Comparative analysis of the sensitivity of these magnitudes (ie. $<r^2>$ $<\mu^2>$, $<\gamma^2>$ and $<_mK>$) to conformational changes in synthetic polymers indicated that usually $<\gamma^2>$and $<_mK>$ are the most sensitive properties, although there could be differences depending upon the nature of the polymer studied (TONELLI 1977; RIANDE et al. 1980). In the case of polypeptides (LOPEZ-PIÑEIRO et al. 1981; 1983 ; LOPEZ-PIÑEIRO and SAIZ 1983) $<\mu^2>$is more sensitive than $<\gamma^2>$and this more than $<r^2>$ to the conformational character-

istics of the polymers although none of those magnitudes is particularly sensitive to these properties. It seemed then interesting to calculate $<{}_mK>$, that combines the dipole moment vector and the optical anisotropy tensor, and to study its sensitivity to the kind of aminoacid residues and the sequence, in the case of heteropolypeptides, of a given polymer.

As in the preceding papers, the lack of experimental results is a great restrain for our theoretical study. However, the results that we report have been obtained with a consistent set of parameters that, as far as possible, were checked against experimental values for some magnitudes, so we are confident on their applicability. Therefore, we expect our result to be realistics, at least for comparison of values of different polypeptides, wich is the aim of this paper.

MODEL OF CALCULATION

The Kerr constant of a given molecule governs the birefringence produced by an electric field in a sample of independent and uncorrelated molecules; we define its molar value as the difference between the molar polarizations parallel and perpendicular to the electric field $\Delta\alpha_E$ divided by the square of the strenght of the local feld(*) E_{eff}. Thus disregarding the term on hyperpolarizabilities (SUTER and FLORY 1977):

$$ {}_mK = (4\pi N/3E_{eff}^2)\Delta\alpha_E = (2\pi N/15kT)\{(\underline{\mu}^T\underline{\underline{\hat{\alpha}}}\underline{\mu}/kT)+|(\varepsilon-1)/(\tilde{n}^2-1)|\,Tr(\underline{\underline{\hat{\alpha}}}\hat{\alpha})\} \qquad (1) $$

where N is Avogadro's number; k is the Boltzmann constant; ε is the static dielectric constant of the medium; $\tilde{n}$ its refractive index at the wavelength at which the meausrement is carried out; $\underline{\mu}$ is the permanent dipole moment and $\underline{\mu}^T$ is its transpose; $\underline{\underline{\hat{\alpha}}}$ is the anisotropic part of the polarizability tensor. For molecules with large dipole moment such as polypeptides, the term on dipole moment is much larger than that on optical anisotropy (INGWALL et al. 1973) thus this second term may be disregarded and Eq. 1 rewritten as:

$$ {}_mK = (2\pi N/15\{kT\}^2)\underline{\mu}^T\underline{\underline{\hat{\alpha}}}\underline{\mu} \qquad (2) $$

Assuming that both $\underline{\mu}$ and $\underline{\underline{\hat{\alpha}}}$ of the polymer may be obtained by addition of contributions from each repeating unit (MEYER and OTTERBEIN 1931), the value of $\underline{\mu}^T\underline{\underline{\hat{\alpha}}}\underline{\mu}$ of a polimeric chain comprising x units in a given conformation may be obtained by serial multiplication of generator matrices $\underline{\underline{Q}}_i$ (FLORY 1969; 1974) and therefore:

$$ {}_mK = (2\pi N/15\{kT\}^2)\prod_{i=1}^{x}\underline{\underline{Q}}_i \qquad (3) $$

In the case of polypeptides, the average of Eq. 3 over all the conformations of the chain may be performed for each single repeating unit

(*) Molar Kerr constants defined on this basis differ from those used by some other authors (LE FEVRE 1972) by a factor of 9.

(INGWALL et al.1973), and Eq. 3 can be rewritten as:

$$<_m K> = (2\pi N/15\{kT\}^2) \prod_{i=1}^{x} <\underline{\underline{Q}}>_i \quad (4)$$

where the average matrix $<\underline{\underline{Q}}>_i$ is obtained by replacing the terms of $\underline{\underline{Q}}_i$ with their corresponding averages over the rotations ϕ_i, ψ_i of the skeleton and χ_i of the lateral group (see Fig. 1).

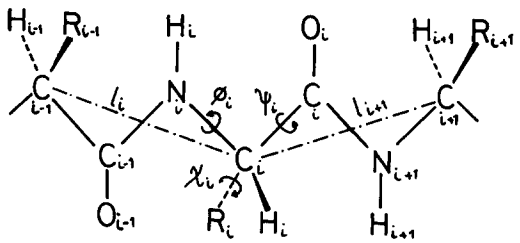

Fig. 1: A segment of an α-polypeptide in its planar all trans conformation. Virtual bonds connecting consecutive α carbons are shown by dashed dotted lines.

As in the calculation of other conformational properties, we have assumed that all the bonds of the lateral groups beyond the C^{α}- $C^{\beta}(\chi_i)$ are placed at their minimun energy as determined by MOMANY et al. (1975a). Conformational energies were calculated with the ECEPP program (MOMANY et al. 1975b) using the empirical functions propossed by MOMANY et al. (1975a). The computation of conformational energies and the calculation of averages are described by LOPEZ-PIÑEIRO et al. (1981). All the averages were performed at 25°C.

Contributions $\underline{\underline{\hat{\alpha}}}_i$ of each repeating unit to the optical anisotropy tensor of the chain were determined by addition of optimized bond contributions as detailed by LOPEZ-PIÑEIRO et al. (1983).

The evaluation of the dipole moment of each repeating unit $\underline{\mu}_i$ was described by LOPEZ-PIÑEIRO and SAIZ (1983). $\underline{\mu}_i$ was calculated as the sum of two contributions, one from the skeleton and a second one from the lateral group using a program MINDO/3 (DEWARD and HASELBACH 1970) to compute these two contributions.

R E S U L T S A N D D I S S C U S S I O N

a) Model Compounds

Experimental values of $<_m K>$ of N-acetyl-N'-methyl amides of glycine, alanine and leucine are reported in the literature (INGWALL et al. 1973). Table I compares our theoretical results with the experimental values and with the results calculated by some other authors(KHANARIAN et al. 1981). As it can be seen from this Table, the agreement with experience is excellent in the cases of Gly and Leu and very poor for Ala. The fact that theoretical values for Ala are in disagreement with experimental results, not only in our case but also in the calculations reported before (INGWALL et al. 1973; KHANARIAN et al. 1981), is not just a coincidence. As it has been pointed

out (INGWALL et al. 1973), $_{m}K$ for fixed conformations of Ala is small and negative within a region close to the minimun of energy; however it increases steeply reaching large positive values even in areas where the conformational energy is not very high. Under those conditions, small modifications on the conformational energies within the limits of reliability of the method of calculation have large effects on the results for the averaged $<_{m}K>$; thus, the experimental result may be reproduced with a small adjustment of the energies in a region close to the minimun. In our case, agreement between theory and experiment is achieved by rising the energies of the conformations defined by $252<\psi<344$ and $18<\phi<108$ by an amount $\Delta E = 0.5$ kcal mol^{-1} (see Table I).

TABLE I

Kerr constants of N-acetyl-N'methyl amides of Glycine, Alanine and Leucine $<_{m}K>(10^{-27}V^{-2}m^{5}mol^{-1})$

Compound	This work	Literature calc.	Literature exp.[a]
Gly	157	159[a] ; 177[b]	167
Ala	36 ; 127[c]	18[a] ; 64[b] ; 127[a]	128
Leu	115	4[a], 30[a]; 127[a]; 145[a]	117

a: INGWALL et al. 1973; b: KHANARIAN et al. 1981; c: calculated with the modified conformational energies; $\Delta E = 530$ cal mol^{-1}; see text.

b) Homopolypeptides

Eq. 4 was used to compute $<_{m}K>$ for homopolypeptides of the twenty natural α-aminoacids having the structural formula $CH_3-(CO-NH-CHR)_{x-1}-NH-CH_3$ with degrees of polymerization x ranging from 1 to 300. Ratios $K_x = <_{m}K>/x$ were evaluated for each x and extrapolated to $x \to \infty$ by plotting K_x versus $1/x$ and projecting the linear part; typical differences between K_{300} and K_∞ are of the order of 5-10%. Fig. 2 shows the values of K_∞ for the twenty homopolypeptides.

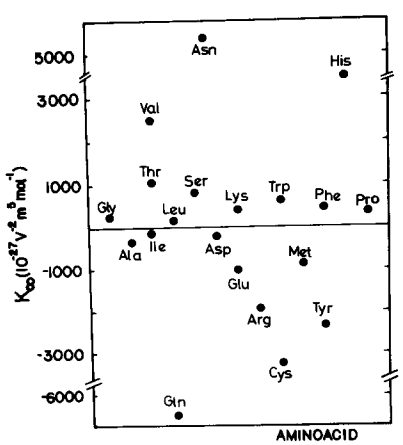

Fig. 2: Values of K_∞ for homopolypeptides of the twenty natural α-aminoacids.

The values of K_∞ for the twenty homopolymers range from 5613 to -6053 in $10^{-27}V^{-2}m^5mol^{-1}$units, although for most of them are comprissed between $\pm 114\times 10^{-27}V^{-2}m^5mol^{-1}$. This variation is much larger than that of either dimensions, dipole moments or optical anisotropies. Therefore, the Kerr constant is much more sensitive to the kind of aminoacid of the polypeptides than any of the other three properties.

The most important features on Fig.2 can be easily explained by taking into account that, as Eq. 2 shows, ${}_mK$ is proportional to the product $\underline{\mu}^T\underline{\underline{\hat{\alpha}}}\underline{\mu}$. If the $\underline{\underline{\hat{\alpha}}}$ tensor of a given molecule is diagonalized and its dipole moment $\underline{\mu}$ is written in the coordinate system in which $\underline{\underline{\hat{\alpha}}}$ is diagonal, that product takes the form :

$$\underline{\mu}^T\underline{\underline{\alpha}}\underline{\mu} = \mu_x^2\,\hat{\alpha}_{xx} + \mu_y^2\,\hat{\alpha}_{yy} + \mu_z^2\,\hat{\alpha}_{zz} \qquad (5)$$

and therefore, ${}_mK$ (and then K_∞) increases as the components of $\underline{\mu}$ and $\underline{\underline{\hat{\alpha}}}$ increase. Among these two factors, $\underline{\mu}$ is the most important one since it appears squared. One would then expect K_∞ to increase (in absolute value) as the optical anisotropy ratio G_∞ (governed by $\underline{\underline{\hat{\alpha}}}$) and mainly the dipole moment ratio D_∞ (governed by $\underline{\mu}$) increase. That explains for instance the very large absolute values of K_∞ for Asn and Gln that have high values of D_∞ and G_∞ (LOPEZ-PIÑEIRO et al. 1983). Moreover, Pro exhibits low values of D_∞ and G_∞ and its K_∞ is very small in absolute value; Trp has large G_∞ but a very low value of D_∞ and its resulting K_∞ is small.

This simple relationship between D_∞, G_∞ and K_∞ gives only an estimate of the absolute value of K_∞, but not its sign. Besides, it fails in some cases, as for instance with Ile that despite of having a very large value of D_∞ presents the smallest value of K_∞ among all the homopolypeptides. These failures arise from the fact that, by using D_∞ and G_∞ , we are considering only the absolute values of $\underline{\mu}$ and $\underline{\underline{\hat{\alpha}}}$, but not their signs. Since $\underline{\underline{\hat{\alpha}}}$ is a traceless tensor, $\hat{\alpha}_{xx} + \hat{\alpha}_{yy} + \hat{\alpha}_{zz} = 0$, and one of the components should have a sign opposite to the other two. The components μ_x, μ_y and μ_z appear squared, and therefore, their signs are irrelevant, but, depending upon their relative value (i.e., depending on the orientation of $\underline{\mu}$ relative to the system in which $\underline{\underline{\hat{\alpha}}}$ is diagonal), the term $\underline{\mu}^T\underline{\underline{\hat{\alpha}}}\underline{\mu}$ may be large and positive if large components of $\underline{\mu}$ combine which large and positive components of $\underline{\underline{\hat{\alpha}}}$ or negative if the large $\underline{\mu}$'s combine with large and negative $\underline{\underline{\hat{\alpha}}}$'s, and, even there may be a substantial cancellation among the three terms of Eq. 5. Unfortunately, there is not an easy way of predicting the sign of K_∞ or even to ascertain whether or not the proportionality between K_∞, D_∞ and G_∞ will be true from the values of these two last magnitudes, or even from the

values of the contributions $\underline{\mu}_i$ and $\underline{\hat{\alpha}}_i$ of the repeating units to the dipole moment and optical anisotropy tensor of the chain, since the product $\underline{\mu}^T\underline{\hat{\alpha}}\underline{\mu}$ is positive for a single residue of any of the twenty α-aminoacids, and it is only for chains with x≃4-5 when the changes in sign start to appear.

The only values of $<_mK>$ for homopolypeptides that we have found in the literature are the results of theoretical caluclations reported by Flory and coworkers (INGWALL et al. 1973) that are compared with our own values in Table II. There are substantial discrepancies between these results that arise from two factors, namely differences in the contributions $\underline{\mu}_i$ and $\underline{\hat{\alpha}}_i$ of the repeating units, and differences in conformational energies. This last kind of differences may be removed by assuming free rotation over the φ and ψ angles; the results thus obtained (last row in Table II) differ only in about 10%.

TABLE II

Comparison of our results of Kerr constant with literature values

$<_mK>/x_{x=15}$ $(10^{-27}V^{-2}m^5mol^{-1})$

Polymer	This work	Literature
Polyglycine	39	78[a]
Polyalanine	-7 ; 21[c]	4[a] ; 44[b]
Free rotation	343	306

a: BRANT et al. 1967; b: INGWALL et al. 1973; c: calculated with the modified conformational energies, ΔE= 530 cal mol^{-1} (see text).

c) Heteropolypeptides

Eq. 4 can also be used to calculate $<_mK>$ of heteropolypeptides with the only modification that $<\underline{Q}>_i$ may then be different for each unit. Tables III and IV summarize the results of K_x that we have obtained for some enzymes and proteins (calculated for their respective random coil states) whose aminoacid sequences have been established before.

TABLE III

Calculated values of Kerr constant for some enzymes

Enzyme	Sequence	$K_x(10^{-27}V^{-2}m^5mol^{-1})$
Bradykinine	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	629
Bovine Oxytocin	Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly	-316
Bovine Vasopressin	Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly	72

Differences in the value of K_x for the enzymes are much larger than in the case of dimensions, dipole moments or optical anisotropies. It is interesting to point out that the structural differences between Oxytocin and Vasopressin are the substitution of Ile by Phe in position 3 and Leu by Arg in position 8; these variations produce a large effect in the values of K_x that change from -316 in Oxytocin to 72 in Vasopressin (in $10^{-27}V^{-2}m^5mol^{-1}$ units).

TABLE IV

Values of Kerr constant of some proteins

K_x ($10^{-27}V^{-2}m^5mol^{-1}$)

Protein	Actual sequence	Random sequence	Standard error	Polymerization degree
Myoglobin	-65	34	17	151
Lysozyme	-341	80	47	129
Chymotripsinogen	-155	173	34	245
Ribonuclease	39	-236	83	124
Human Hemoglobin α	355	138	73	141
Human Hemoglobin β	14	139	44	146
Human Hemoglobin γ	-76	-31	60	146
Human Hemoglobin δ	-278	11	63	147
Horse Hemoglobin α	432	237	39	141
Horse Hemoglobin β	-4	34	45	146

Table IV shows the values of K_x calculated for ten proteins in the random coil state. The second column of this Table represents the results obtained with the actual sequence of each protein; it is interesting to notice that the values of K_x range from about 400 to -300 in $10^{-27}V^{-2}m^5mol^{-1}$ units, a variation much larger than that of dimensions, dipole moments or optical anisotropies that allows to distinguish all the proteins by their respective values of K_x. The third column of Table IV summarizes the results obtained averaging ten chains generated with random sequences for each protein maintaining the same aminoacid contents. Standars errors of this calculation are shown in the fourth column; the large values of these standard errors indicate that the calculated K_x's were quite different for each of the ten chains and, therefore, that this magnitude is very sensitive to the sequence of aminoacids of the chain. The same conclusion is obtained by comparison of the second and third columns; the value of K_x changes substantially if the sequence of aminoacids is modified.

The great sensitivity of K_x to the sequence also appears in short chains of two or three aminoacid residues. For instance, if we take the pair Asn, His and calculate K_x for the molecule CH_3-CO-NH-CHR-CO-NH-CHR'-NH-CH_3, where R is the lateral group of Asn and R' that of His, we obtain K_x= -1007, whereas if the sequence is reversed, i.e., for the molecule CH_3-CO-NH-CHR'-CO-NH-CHR-NH-CH_3, the value of K_x is 3854 , both in $10^{-27}V^{-2}m^5mol^{-1}$units. In the case of trimers, if we take for instance the set Ala, Ser, Asp, the different sequences give values of K_x ranging from 309 to -395 in the same units.

We therefore conclude that the Kerr constant seems to be a very good probe for studying conformational properties of polypeptides. It would be interesting to carry out some experimental measurements in order to check out the validity of our theoretical results.

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