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STEREOCHEMICAL CRITERIA FOR POLYPEPTIDES AND PROTEINS

VI. NON-BONDED ENERGY OF POLYGLYCINE AND POLY-L-ALANINE IN THE CRYSTALLINE β-FORM*

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SUMMARY

This paper describes calculations on the packing energy in the crystal structures of β-forms of polyglycine and poly-L-alanine. It is shown that the non-bonded interactions between β-chains, in a pleated sheet structure, yields a minimum energy when the interchain spacing is about 4.8 Å, which is well-suited for the formation of interchain NH $\cdots$ O hydrogen bonding. The attempts made to deduce the crystal structures of β-forms of polyglycine and poly-L-alanine are also described.

INTRODUCTION

The description of conformations of polypeptide chains have been greatly facilitated by the introduction of stereochemical parameters associated with the polypeptide chain, such as dihedral angles of rotation, bond angles *etc.* Denoting the dihedral angles of rotation about N–C^{α} and C^{α}–C′ bonds by φ and ψ, RAMACHANDRAN and co-workers[1,2] classified the conformations of a pair of peptide units as 'allowed' and 'disallowed' by studying the restrictions imposed on φ and ψ due to limiting contact distances between the two peptide units. The result of the analysis was a (φ, ψ) conformational map showing the allowed and disallowed regions enclosed by certain contours. Since then, such conformational maps (using both hard-sphere model[1,2] as well as potential functions[3,4] to evaluate the consequences of non-bonded interactions) have been in vogue and have proved very useful in studying polypeptide and protein chain conformations**. But this is not the whole story, since only interactions within a single polypeptide chain have been taken into account while preparing these conformational maps. Though it is very unlikely that a conformation shown as disallowed in a (φ, ψ) map owing to bad intrachain contacts will be realized as a stable conformation

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** The first studies on the application of energy criteria on peptide structures were made by LIQUORI *et al.*[5].

when the interchain interactions are very favourable, the different allowed conformations in the map may be further stabilized or destabilized by interchain interactions to varying extents. For example, the conformational maps give a low energy for polyglycine when it takes an α-helical form. But polyglycine does not occur in the α-helical form. The reason for this rests partly on the fact that the two forms of polyglycine, polyglycine I and polyglycine II, though not lying in any deep potential minimum in a (φ, ψ) map for a single chain, are considerably stabilized by non-bonded interactions and hydrogen bonds between different chains. Evidence for favourable non-bonded interchain interactions is obtained by energy calculations on the packing of polyglycine I and of β-poly-L-alanine and is discussed in this paper. The favourable nature of the van der Waals energy of the polyglycine II structure, apart from the regular occurrence of hydrogen bonds, has already been discussed[7].

RESULTS AND DISCUSSION

The pleated sheet structures

The completely extended conformation of a polypeptide chain corresponds to the case $\varphi = 0°$, $\psi = 0°$. The distribution of intrachain non-bonded interactions in the (φ, ψ)-plane shows a slightly larger energy at $\varphi = 0°$, $\psi = 0°$ than in the neighbourhood of $\varphi = 60°$, $\psi = 330°$. This indicates that the fully extended conformation is less likely to occur than a slightly puckered one obtained rotating slightly about the single bonds N–C^{α} and C^{α}–C'. Putting several such chains side by side, one obtains the pleated-sheet conformation. The parallel and antiparallel chain pleated sheet structures proposed by PAULING AND COREY[8] consist of polypeptide chains in such a nearly fully extended conformation arranged to form a sheet, the chains within each sheet being held together by means of NH · · · O hydrogen bonds between the chains. The distance between the chains in a sheet determines the hydrogen bond lengths, N · · · O, and the optimum distance for good hydrogen bonds, worked out by PAULING AND COREY[8], are 4.85 and 4.75 Å, respectively, for parallel and antiparallel chain pleated sheet structures. In this context it would also be interesting to determine the influence of interchain non-bonded interactions on the spacing between the chains in a sheet. The variation of potential energy of the interchain interactions with the spacing of the chains has therefore been worked out for parallel and antiparallel chain pleated sheets using a potential function of the form

$$V = ae^{-\mu r} - c/r^6$$

to evaluate the energy due to non-bonded interactions between a pair of atoms at a distance r (Å) apart. The values of parameters a, μ and c were arrived by SCOTT AND SCHERAGA[4] and were modified by BRANT AND FLORY[6]. The actual values used in the present study were those adopted by RAMACHANDRAN, VENKATACHALAM AND KRIMM[3] based on the data in ref. 6.

Table I shows the variation of non-bonded energy with spacing for polyglycine and poly-L-alanine. For polyglycine, the potential energy is a minimum for a distance of separation of 4.75 Å for both parallel and antiparallel sheets, while in the case of poly-L-alanine it is a minimum at 4.75 Å for the parallel and 4.65 Å for the antiparallel structure. In general, the potential energy shoots up appreciably for distances of separation less than 4.55 Å, while the variation in the potential energy is not appreci-

TABLE I

VARIATION OF NON-BONDED ENERGY WITH DISTANCE OF SEPARATION BETWEEN A PAIR OF POLYPEPTIDE CHAINS IN THE PARALLEL AND ANTIPARALLEL PLEATED SHEET ARRANGEMENTS

Separation (Å)	*Parallel pleated sheet (energy in kcal/mole/residue)*		*Separation (Å)*	*Antiparallel pleated sheet (energy in kcal/mole/residue)*	
	Polyglycine	*Poly-L-alanine*		*Polyglycine*	*Poly-L-alanine*
4.35	−3.77	−5.12	4.25	−0.56	−2.47
4.45	−5.26	−6.52	4.35	−3.37	−5.21
4.55	−6.06	−7.23	4.45	−4.91	−6.66
4.65	−6.41	−7.50	4.55	−5.67	−7.34
4.75	−6.49	−7.50	4.65	−5.97	−7.56
4.85	−6.40	−7.34	4.75	−6.00	−7.52
4.95	−6.23	−7.11	4.85	−5.89	−7.33
5.05	−6.01	−6.83	4.95	−5.70	−7.08
5.15	−5.78	−6.54	5.05	−5.48	−6.79
5.25	−5.54	−6.26	5.15	−5.24	−6.50
5.35	−5.32	−5.99	5.25	−5.01	−6.23

able in the range 4.65 to 4.80 Å. Therefore, it may be concluded that non-bonded interactions also favour a value for the distance of separation, which is suitable for interchain NH···O hydrogen bonding. It is also clear that the interchain separation within the sheets is rigidly controlled more by hydrogen bonding than by non-bonded interactions. Moreover, so far as the sheet structure alone is concerned, poly-L-alanine has a lower energy than polyglycine. It may also be pointed out that, considering only non-bonded interactions, parallel and antiparallel chain pleated sheets are about equally likely, having closely similar values of stabilizing energy.

Crystal structures of the β-form of polyglycine and poly-L-alanine

The crystal structures of the above two polypeptides contain a three-dimensional arrangement of pleated sheets, in which they are stacked one over the other. The fact that the chains are not in the fully extended form is evident from the fibre repeat of about 7 Å that one obtains from X-ray photographs of the β-forms of polyglycine and poly-L-alanine. This should be compared with a value of about 7.2 Å which would be expected for fully extended chains. Since the repeat for the antiparallel pleated sheets is of the right order (= 7 Å), the chains have been taken to be in the antiparallel arrangement for the purpose of calculating the energy of the three-dimensional structures.

Assuming the structure to consist of antiparallel pleated sheets, the distance between the chains within the sheets was kept at a constant value of 4.75 Å. The distance d between the sheets is largely governed by the side chains involved. Furthermore, while stacking the sheets one over the other, it is possible to slide the sheets over one another in a direction parallel to the hydrogen bonds and perpendicular to the length of the chains. A typical arrangement is sketched in Fig. 1. As a result of sliding a sheet over the other, the angle γ is not necessarily equal to 90°, and will have to be explicitly considered. The calculations described below were an attempt to compute

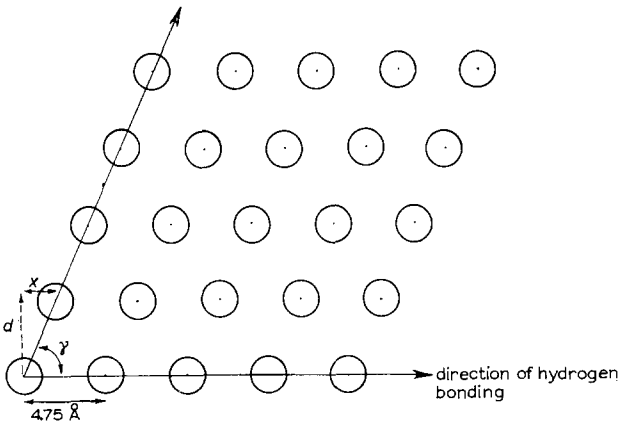

Fig. 1. Three-dimensional arrangement of polypeptide chains in β-conformation. The direction of the progress of the chains is normal to the plane of the paper.

optimum values for this angle γ and the side chain spacing d for polyglycine I and β-poly-L-alanine making use of potential functions described earlier.

Potential energy calculations were done by first fixing the distance d and starting with the position corresponding to $\gamma = 90°$. The sheets were then shifted by a magnitude, x Å, along the direction of the hydrogen bonds. For each shift, the total energy of non-bonded interaction was computed by generating five such sheets and calculating the potential energies of interaction of the atoms in these sheets with the atoms in a typical peptide unit at the centre of the volume considered. Thus, all interactions between atoms at distances of less than 7–8 Å were included. The value of γ corresponding to each packing configuration is given by

$$\gamma = \cos^{-1}[x/\sqrt{x^2 + d^2}]$$

Polyglycine I has been studied by ASTBURY[9] with the use of powder diagram of the β-material. He has assigned a value of 3.45 Å for the spacing between the sheets and γ was deduced to be 66°. Poly-L-alanine was obtained in a highly oriented form by BAMFORD and co-workers[10,11] who have shown that $d = 5.35$ Å and $\gamma = 66°$ yielded good agreement with X-ray fibre pattern.

In view of these observations, in the calculations, the distance d between the antiparallel sheets was kept at a fixed value of 3.45 and 5.35 Å for polyglycine and poly-L-alanine respectively. For both cases, the potential energy calculations were performed for values of x varying from -4.5 to $+4.5$ Å at intervals of 0.5 Å. The variation of the non-bonded packing energy with lateral shift is given in Table II for polyglycine I and β-poly-L-alanine.

It may be seen that, for polyglycine I, there are two conformations for which the energy takes a local minimum value. One occurs around $x = -3.0$ Å (with $\gamma = 131°$) having a minimum of -15.8 kcal/mole per residue and the second at $x = 1.5$ Å ($\gamma = 67°$) with a value of -15.9 kcal/mole per residue for the energy. The conformation for which the second minimum occurs agrees perfectly with the conformation assigned to polyglycine I by ASTBURY[9]. The minimum at $\gamma = 131°$ corresponds to a conformation which has not been observed for polyglycine I and this seems to require further study.

TABLE II

VARIATION OF THE ENERGY OF INTERCHAIN INTERACTIONS WITH THE SHIFT x OF THE PLEATED SHEETS OVER ONE ANOTHER

x (Å)	*Polyglycine* (d = 3.45 Å)		*Poly-L-alanine* (d = 5.35 Å)	
	γ	V (kcal/mole/residue)	γ	V (kcal/mole/residue)
−4.5	142.5°	−14.46	130.1°	−12.28
−4.0	139.2°	−15.08	126.8	−12.22
−3.5	135.4°	−15.59	123.2°	−12.13
−3.0	131.0°	−15.76	119.3°	−12.00
−2.5	125.9°	−15.69	115.0°	−11.91
−2.0	120.1°	−15.49	110.5°	−11.90
−1.5	113.5°	−15.13	105.7°	−12.00
−1.0	106.2°	−14.62	100.6°	−12.16
−0.5	98.2°	−14.53	95.3°	−12.31
0.0	90.0°	−15.08	90.0°	−12.39
0.5	81.8°	−15.68	84.7°	−12.43
1.0	73.8°	−15.93	79.4°	−12.44
1.5	66.5°	−15.93	74.3°	−12.45
2.0	59.9°	−15.84	69.5°	−12.44
2.5	54.1°	−15.74	65.0°	−12.42
3.0	49.0°	−15.64	60.7°	−12.42
3.5	44.6°	−15.45	56.8°	−12.43
4.0	40.8°	−14.99	53.2°	−12.40
4.5	37.5°	−14.44	49.9°	−12.34

On the other hand, in the case of poly-L-alanine, there is only one minimum and this occurs at $x = 1.5$ Å ($\gamma = 74°$) with an energy value of −12.45 kcal/mole per residue. Thus, in this case also, there is fairly good agreement between prediction based on energy calculations and experimental value of $\gamma = 66°$.

Nevertheless, more elaborate calculations, varying also the intersheet spacing d, failed to show minimum energy for the experimentally observed values for d of 3.45 and 5.35 Å for polyglycine I and poly-L-alanine. The reason for this is not clear. It is found that the minimum energy corresponds to much lower values of intersheet distance d than the values derived from experimental observation.

However, the calculations clearly show that the non-bonded energy of packing takes quite low values of about −16 kcal/mole and −12.5 kcal/mole for β-polyglycine and β-poly-L-alanine for the observed structures. This indicates the β-polyglycine has much lower stabilizing energy than β-poly-L-alanine.

The contribution of packing of polyglycine-II structure to its stability has already been studied[7] and it has been found that with the same potential functions, the energy of interchain interaction was equal to −12.5 kcal/mole, which is clearly about 10 kcal/mole less than the contribution from the intrachain interactions. This demonstrates the importance of interchain interactions for the conformation of polyglycine, apart from the vital contributions from hydrogen-bonding. These calculations indicate a probable explanation of the reason why polyglycine does not occur in an α-helical form.

The calculations also suggest that the β-form of polyglycine is much more stable than polyglycine II. This agrees excellently with the observation[12] that mild mechanical

treatment of polyglycine-II film very easily induces the transformation from polyglycine II to polyglycine I. This easy transformation II → I, namely the β-form, has, in fact, been responsible for difficulty in obtaining well-oriented films or fibres of polyglycine II.

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