Dielectric Effects and Conformations of Small Peptides in Aqueous Solution

By J. BEACHAM, V. T. IVANOV, G. W. KENNER, and R. C. SHEPPARD
(*The Robert Robinson Laboratories, University of Liverpool*)

EVIDENCE from the dielectric increments of some pairs of diastereoisomeric oligopeptides and from differences in yields of cyclisation of diastereoisomeric pentapeptides has been presented[1] in favour of the view that, even in aqueous solution, peptides do not exist in merely random sets of conformations arising from free rotation of the bonds between the planar amide groups and the tetrahedral, asymmetric carbon atoms. We now report dielectric data at three temperatures for all the diastereoisomeric di-, tri-, and tetrapeptides composed entirely of either alanine or serine. In each series the all-L-peptide has the largest dielectric effect, indicating the greatest separation of charge in the dipolar ion. If the amide groups are planar and *trans*, this result is compatible only with peptide conformations defined by $\phi,\phi' = 0—180°$ or $\phi,\phi' = 180—360°$, where ϕ and ϕ' are the dihedral angles of rotation about the N–C_α and C_α–CO bonds, *viz.* Figure 1.

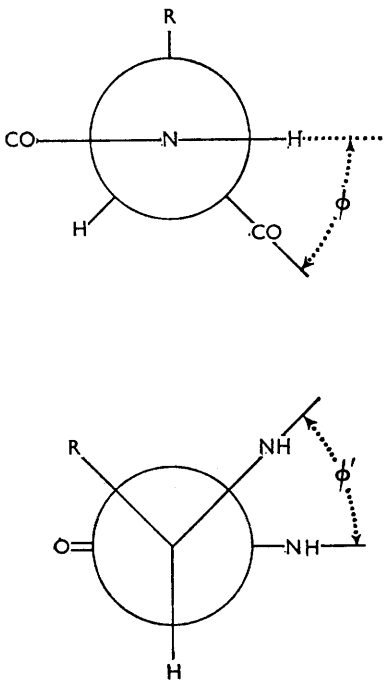

FIGURE 1

Expressed graphically, the favoured conformations are found in the lower left-hand or upper right-hand quadrants of Figure 2. However,

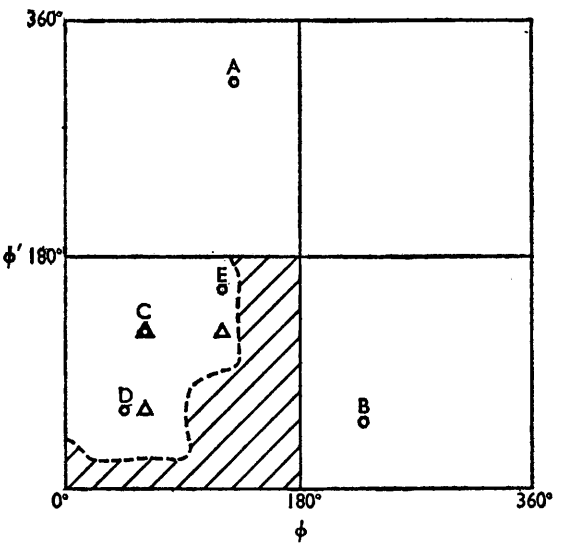

FIGURE 2. *Conformations of peptide chains, with planar* trans*-amide groups, in terms of angles of rotation in the* N–C_α *bonds* (ϕ) *and the* C_α–CO *bonds* (ϕ'). A, *right-handed* α*-helix;* B, *left-handed* α*-helix;* C, *pleated sheet;* D, *threefold helix similar to polyglycine* II; E, *threefold helix as in poly-*L*-proline;* Δ, *three of the seven conformations allowed for alanine peptides according to Némethy and Scheraga.*[4]

recent calculations[2,3,4] have shown that excessive non-bonding interactions arise in all conformations in the upper right-hand quadrant and in part of the lower left-hand quadrant (shaded in Figure 2). The combination of our experimental results with these theoretical calculations thus eliminates, for oligopeptides in aqueous solution, all but a small fraction of the possible conformations, at least as major participants. Notably the α-helical conformations (A, B in Figure 2) are

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[2] G. N. Ramachandran, C. Ramakrishnan, and V. Sasisekharan, *J. Mol. Biol.*, 1963, **7**, 95.
[3] P. de Santis, E. Giglio, A. M. Liquori, and A. Ripamonti, *Nature*, 1965, **206**, 456.
[4] G. Némethy and H. A. Scheraga, *Biopolymers*, 1965, **3**, 155.

excluded, and this emphasises the importance of intramolecular hydrogen bonds in stabilising right-handed α-helices of polypeptides (*cf.* ref. 2). On the other hand, the permitted area includes those conformations which are repeated in the pleated sheet (C), in a threefold helix[3] similar to poly-glycine II (D), and in the threefold helix of poly-L-proline (E). These are similar to three of the seven conformations for alanine residues, allowed by calculations[4] assuming sixfold energy-minimum functions for ϕ and ϕ';[5] the other four conformations are incompatible with the dielectric data.

If the square root of the dielectric effect is taken as a very approximate measure of the distance between chain termini,[6] it is possible to correlate the dielectric effect of each diastereisomer with measurements of molecular models. There is encouraging agreement between the trends of the data and the dimensions of models with the three conformations mentioned above, but the problem is too complex for complete solution in this way because (*a*) a more satisfactory theory connecting dielectric increment with dipole moment is necessary, (*b*) more than one conformation may contribute significantly to the population of conformers. The convergence of values for diastereoisomers with increasing temperature indicates contributions from more than one conformation. Although there are significant differences between the data for analogous alanine and serine peptides, the broad similarity of the effect of optical configuration is impressive and it disposes of the possibility that the existence of preferred conformations is a consequence of the hydrophobic character of the side-chains.

Dielectric effects were measured[1] at four concentrations in the range 0·0005—0·04M for each compound, and the slope of the straight line, obtained by plotting capacitance against molarity of the solution, was compared with that obtained for glycine. The results, for which an average accuracy of 3% is estimated, are therefore expressed in "glycine units" (G.U.), *i.e.* the ratio of the two slopes; multiplication of G.U. at 0·5° by 23·8, or at 30·5° by 22·5, gives the dielectric increment.

The alanine peptides were synthesised from alanine t-butyl ester by carbodi-imide coupling with benzyloxycarbonylalanine. Further residues were added singly by alternate hydrogenolysis and coupling with benzyloxycarbonylalanine 2,4,5-trichlorophenyl ester. The penultimate step was removal of the t-butyl group with 98% trifluoroacetic acid. The optical rotations of the free peptides, recrystallised after hydrogenolysis, agreed with the available published data.[7] The serine peptides were prepared in a generally similar manner *via* derivatives of *O*-t-butylserine,[8] except that carbodi-imide coupling was used throughout and *N*-t-butyloxycarbonyl-*O*-t-butyl-serine was the last addend. All the protecting groups were removed by trifluoroacetic acid and the peptide was liberated by adsorption on Dowex-50 anion-exchange resin and elution in aqueous pyridine.

TABLE 1

Dielectric Effects (Glycine Units) at 28 Mcs. of Alanine Peptides[b] in Aqueous Solution

Temp.°	LL	LD[a]	LLL	LLD[a]	DLL	DLD[a]	LLLL	DLLL	LLLD[a]	DDLL	LLDL	DLLD[a]	LDLL	LDLD[a]
0·5	3·2	2·65	6·0	5·6	5·3	4·3	9·5	8·25	7·9	7·6	6·55	6·35	6·0	5·25
30·5	2·95	2·5	5·9	5·0	4·5	4·05	8·5	7·6	7·2	6·95	5·9	5·9	5·3	4·7
60·0	3·0	2·6	4·9	5·0	4·05	3·7	7·9	7·85	7·4	6·6	5·5	5·9	5·2	4·8

[a]Enantiomer actually used. [b]Diglycine at 0·5° 3·2, at 30·5° 3·2, at 60° 2·95 G.U. Triglycine at 0·5° 5·7, at 30·5° 5·45, at 60° 5·2 G.U. Tetraglycine at 0·5° 7·6, at 30·5° 7·4, at 60° 6·6 G.U.

TABLE 2

Dielectric Effects (Glycine Units) at 28 Mcs. of Serine Peptides in Aqueous Solution

Temp.°	LL	LD	LLL	LLD	DLL	DLD	LLLL	DLLL	LLLD	DDLL	LLDL	DLLD	LDLL	LDLD
0·5	3·0	2·5	5·7	4·9	5·1	4·0	8·8	7·0	7·3	7·4	5·2	6·5	6·0	5·0
30·5	2·8	2·5	5·3	4·9	4·95	4·0	8·0	7·2	7·2	6·9	5·5	6·0	5·4	5·0
60·0	2·5	2·3	5·1	4·2	4·3	3·9	7·3	6·6	6·8	6·45	5·45	5·7	4·8	4·8

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