THE CONFORMATIONAL ENERGY MAP FOR THE DISULPHIDE BRIDGE IN PROTEINS.

D. Perahia and B. Pullman,

Institut de Biologie Physico-Chimique, 13, rue P. et M. Curie, Paris 5è, France.

Received February 12, 1971

Summary. Calculations carried out by the quantum-mechanical PCILO method indicate that the stable conformation of the disulphide bridge corresponds to a twist of 90°-100° around the S-S bond. This result is confirmed by the experimental studies on the conformation of these bridges in a number of cystine derivatives and in the globular proteins:ribonuclease S, chymotrypsin and lysozyme. The calculations predict a higher cis (90° to 0°) than trans (90° to 180°) rotational barrier.

The conformational energy map of the disulphide bridge of proteins was recently investigated theoretically on the model compound $CH_3 - S - S - CH_3$ by Ponnuswamy (1) both by the "empirical" computations and with the help of a simple quantum-mechanical procedure, namely the Extended Hückel Theory. The results of both types of computation were unsatisfactory. Thus, while experimentally the S - S bridges undergo a twist of about 90° (right handed or left handed) around the S - S bond in amino acids and in proteins (vide infra), the "empirical" computations indicate an energy minimum for a rotation of about 35° and the Extended Hückel computations favor the angle of 180° (trans conformation).

In view of this situation, we have reinvestigated theoretically the conformational energy curve of the disulphide bridge using a substantially more refined quantum-mechanical procedure, the PCILO (Perturbative Configuration Interaction using Localized Orbitals) method, elaborated recently in our laboratory (2 and the references given therein) and which has given satisfactory results in the construction of conformational energy maps for a large number of amino acid residues of proteins (3)-(7), as well as for other types of biomolecules : nucleosides and nucleotides (8), disaccharides (9), steroids (10), retinals (11) etc...

This method belongs to the all valence electrons procedures studying simultaneously the σ and π electrons. It takes into account interelectronic repulsions and goes beyond the self-consistent field approximation in the calculation of the ground state energy by incorporating an appreciable fraction of

the correlation energy. Its fundamental idea is to choose a set of reasonable bonding and antibonding orbitals localized on the chemical bonds. Such a set may be constructed on a basis of hybridized atomic orbitals, the bond orbitals being obtained as linear combinations of distinct hybrids taken two by two, each bonding orbital being associated with an orthogonal antibonding orbital. A localized orbital representing a lone pair is described by a single hybrid orbital.

The bonding orbitals are used to construct a fully localized Slater determinant. This determinant represents the zeroth order wave function for the ground state of the system. The antibonding orbitals are utilized to build the excited states and a configuration interaction matrix is considered to be constructed on such a basis of configurations. The lowest eigenvalue and eigenstate, i.e. the energy and the wave function of the ground state of the system are then obtained by a Rayleigh-Schrödinger perturbation expansion truncated after the third order.

As a technical simplification, the principal working hypotheses of the CNDO/2 procedure (12)(13) have been retained, in particular the hypothesis of complete neglect of differential overlap as well as the general parametrization of this procedure.

In the present case the CNDO parameters of Santry and Segal are used for the sulphur atom (14). The calculations take into account the 3s and 3p orbitals of that atom but do not include its 3d orbitals. The fact that such a limited

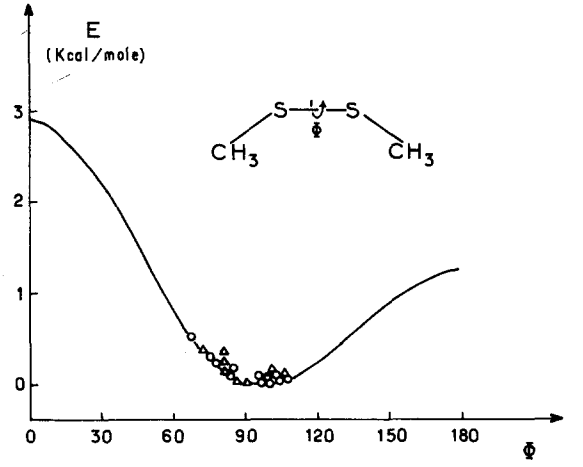

Figure 1. - The conformational energy of CH_3-S-S-CH_3 with respect to the energy of the most stable conformation taken as zero. The drawing in the upper right corner corresponds to $\Phi = 0°$ (cis conformation). Experimental conformations of the S-S bridge in :

- Δ small cystine compounds,
- o globular proteins.

basis set of atomic orbitals is sufficient to account for the main conformational features (in particular for the angle of rotation) in hydrogen persulfide, HS-SH, was shown by the ab initio self-consistent field molecular orbital calculations of Schwartz (15) and Hillier et al. (16), although it is possible that the inclusion of the 3d orbitals may influence the values of the rotation barriers. The geometrical input data are taken following the indications of microwave spectroscopy (17).

Fig. 1 presents the variation of the total molecular energy of dimethyl disulphide, with respect to the energy of the most stable conformation taken as energy zero, for rotations around the disulphide bridge from the cis ($\Phi = 0°$) to the trans ($\Phi = 180°$) position. The rotations were carried out in increments of 30°, except for the zone $\Phi = 60° - 120°$ in which the increments were of 10° in order to locate the minimum more accurately. For the results indicated in the figure, the methyl groups are assumed in the staggered arrangement with respect to the disulphide bridge.

TABLE I

The conformation of the disulphide bridge in cystine derivatives and in globular proteins.

Protein or compound	The residues involved in the bridge	The angle Φ
Ribonuclease-S (18)	26 - 84	101°
	40 - 95	84°
	58 - 110	78°
	65 - 72	84°
Chymotrypsin (19)	1 - 222	113°
	42 - 58	75°
	136 - 201	104°
	168 - 182	67°
	191 - 220	83°
Lysozyme (20)	6 - 127	101.5°
	30 - 115	105.3°
	64 - 80	97.1°
	76 - 94	98.9°
L-cystine dihydrobromide (21)		90°
Hexagonal L-cystine (22)		106°
N,N'-diglycyl-L-cystine dihydrate (23)		101°
Cystine (24)		73.8°
Cystine 2H Br (24)		-81.3°
Cystine 2H Cl (24)		-79.1°
N,N' diglycyl-cystine (24)		-79°
Dimethyldisulphide (microwaves) (17)		84.7°

The most stable conformation is found at Φ = 100° and corresponds thus to an approximately perpendicular orientation of the two methyl groups about the S - S bond. The calculated rotational cis (90° to 0°) and trans (90° to 180°) barriers are 2.9 and 1.3 kcal/mole, respectively.

Similar calculations carried out for the eclipsed arrangement of the methyl groups indicate that the energy minimum is located at the same value of Φ but 1 kcal/mole below the minimum of the staggered form. The trans rotation barrier is similar (1 kcal/mole) but the cis one is much higher 10 kcal/mole).

Fig. 1 confronts also the theoretical results with the X-ray determined conformations of the S-S bridge in a few simple cystine derivatives and in the three globular proteins : ribonuclease S, chymotrypsin and lyzosyme. The corresponding numerical data are indicated in Table I. The overall agreement between theory and experiment is satisfactory, practically all the observed residues occuring within 0.5 kcal/mole above the minimum.

References.

1. Ponnuswamy P.K., Thesis, University of Madras, India (1970).
2. Diner S., Malrieu J.P., Jordan F. and Gilbert M., Theoret. Chim. Acta, 15, 100 (1969).
3. Maigret B., Pullman B. and Dreyfus M., J. Theor. Biol. 26, 321 (1970).
4. Pullman B., Maigret B. and Perahia D., Theoret. Chim. Acta, 18, 44 (1970).
5. Maigret B., Pullman B. and Caillet J., Biochem. Biophys. Res. Comm. 40, 808, (1970).
6. Maigret B., Perahia D. and Pullman B., J. Theoret. Biol. 29, 275 (1970).
7. Perahia D., Maigret B. and Pullman B., Theoret. Chim. Acta, 19, 121 (1970).
8. Berthod H., and Pullman B., Biochim. Biophys. Acta, in press.
9. Giacomini M., Pullman B. and Maigret B., Theoret. Chim. Acta, 19, 347 (1970).
10. Caillet J. and Pullman B., Theoret. Chim. Acta, 17, 377 (1970).
11. Langlet J., Pullman B. and Berthod H., J. de Chim. Phys. 67, 480 (1970).
12. Pople J.A. and Segal G.A., J. Chem. Phys. 43, S 136 (1965).
13. Pople J.A. and Segal G.A., J. Chem. Phys. 44, 3289 (1966).
14. Santry D.P. and Segal G.A., J. Chem. Phys. 47, 158 (1966).
15. Schwartz E., J. Chem. Phys. 51, 4182 (1969).
16. Hillier I.H., Saunders V.R. and Wyatt J.F., Trans. Faraday Soc. 66, 2665 (1970).
17. Sutter D., Dreizler H. and Rudolph H.D., Z. Naturforsch. 20a, 1676 (1965).
18. Wyckoff H.W., Tsernoglou D., Hanson A.W., Know J.R., Lee B. and Richards F.M., J. Biol. Chem. 245, 305 (1970).
19. Birktoft J.J., Matthews B.W. and Blow D.M., Biochem. Biophys. Res. Comm. 36, 131 (1969).
20. Evaluated from crystallographic data kindly communicated by Dr. Blake, See also Phillips D.C., Proc. Natl. Acad. Sci. U.S., 57, 484 (1967).
21. Peterson J., Steinrauf L.K. and Jensen L.H., Acta Cryst. 13, 104 (1960).
22. Oughton B.M. and Harrison P.M., Acta Cryst. 12, 396 (1959).
23. Yakel H.L. and Hughes E.W., Acta Cryst. 7, 291 (1954).
24. From a compilation by Lakshminarayanan A.V., Sasisekharan V. and Ramachandran G.N., in Conformation of Biopolymers (Ed. Ramachandran G.N.) p. 61, Academic Press, vol. 1 (1967).