

PROTON MAGNETIC RESONANCE STUDY OF SOLID L-GLUTAMIC ACID AT ROOM TEMPERATURE

S. C. MISHRA AND R. C. GUPTA

Department of Physics, Lucknow University, Lucknow, India

THE present study of l-glutamic acid is oriented towards the check of the crystal structure¹ and making sure the Zwitterion Structure at room temperature as modified by Hirokawa.

CRYSTAL DATA AND HYDROGEN BONDING IN L-GLUTAMIC ACID

Bernal² reported the crystal structure of l-glutamic acid to have cell dimensions $a = 7.06$; $b = 10.3$ and $c = 8.75$ Å, space group P2₁2₁2₁, and $z = 4$ orthorhombic in form. E.D.P. studies of Hirokawa¹ presented a new modification of the structure in which cell dimensions $a = 5.7$, $b = 17.34$ and $c = 6.95$ Å and $z = 4$ with space group P2₁2₁2₁, and orthorhombic in form. The infrared studies³⁻⁵ associate a zwitterion structure to l-glutamic acid. The cyanoethylation and microwave studies^{6,7} also show the zwitterion properties of this compound. The carbon chain configuration is not coplanar and the structure consists of molecules tied into infinite chain along the direction of the b -axis by O-H...O hydrogen bonds and these chains are linked together in other direction by N-H...O hydrogen bond. In the same molecule H atom is located at a distance of 1.09, 1.01 and .97 Å from C, N and O atoms respectively. The distance between two oxygen atoms of the two carboxyl groups in the molecule is 2.54 Å. The structure of l-glutamic acid gives information regarding the atomic arrangement in a polypeptide chain and the way in which these chains occur in natural proteins. The structure of the chain folded poly (l-glutamic acid)⁸ single crystals accounts for the principal features of the chain.

It is concluded⁹ that in the study of the conformational analysis of solid amino acids, the variations in the bond lengths and bond angles can be nearly neglected. The structure of l-glutamic acid is an example of the fact that while the bond distances and valency angles in the carbon chain of different amino acids remain approximately unchanged, each amino acid assumes a different configuration with respect to a particular C-C bond. The hydrogen bonds play an important role in the configuration of the polypeptide chain and its relation to neighbouring atom. In solutions l-glutamic acid occurs in helical conformation^{10,11}.

EXPERIMENTAL DETAILS

The NMR spectrum of the high purity sample of l-glutamic acid was recorded at room temperature with a 12 inch magnet system at a resonance frequency of 7.5 Mc/S at T.I.F.R. (Bombay). The details of the spectrometer are given elsewhere¹².

CALCULATIONS

Theoretical Second Moment.—There are two contributions to rigid lattice second moment, the S_1 , called intra-molecular, arising due to the interaction between the protons of the same molecule and, S_2 , the inter-molecular, arising due to the interaction between the protons of the different molecules situated at the various lattice sites. The evaluation of S_1 is made by the Van Vleck's formula¹³ for the powder sample.

$$S_1 = \frac{716.55}{N} \sum_{j>k} r_{jk}^{-6} \quad (i)$$

where N is the number of protons in the molecule and r_{jk} is the inter-nuclear distance between the j th and k th nuclei. The modified molecular model of Hirokawa¹ was used according to which cell dimensions are $a = 5.7$; $b = 17.34$ and $c = 6.95$ Å and $z = 4$, with space group P2₁2₁2₁, and orthorhombic in form. The value of S_1 was computed to be 11.99 gauss².

The inter-molecular contribution S_2 to rigid lattice second moment arises out of the interaction between the protons of the different molecules deposited at the various lattice sites and exact evaluation of this contribution is too tedious. An approximate estimation can be made following the method of Andrew and Eades¹⁴ and the value of S_2 was found to be 6.00 gauss². This value appears to be quite reasonable as Agarwal *et al.*¹⁵ obtained this value 6.3 gauss² in case of solid L-alanine, a similar compound. At this point it can be mentioned that further check of the value of S_2 can be made by the formula of Smith¹⁶. Thus the value of the theoretical rigid lattice second moment is then $11.99 + 6.00 = 17.99$ gauss².

Experimental Second Moment.—The experimental value of the second moment are calculated from the NMR line shape derivative $g(H)$ using expression

$$S = \frac{1}{2} \left[\frac{\int_0^\infty g(H) (H-H_0)^3 dH}{\int_0^\infty g(H) (H-H_0) dH} \right] - \frac{1}{2} H^2 m \quad (ii)$$

where H_0 is the value of field at resonance and Hm is sinusoidal field modulation amplitude. The second term in the expression occurs due to suggestions of Andrew¹⁷. The accuracy in the measurement is about ± 1.0 gauss². The experimental second moment at room temperature comes out to be 16.3 gauss². Due to experimental difficulty, investigations of the sample at different temperatures could not be carried out.

RESULTS AND DISCUSSION

The observed value of second moment at room temperature is 16.3 gauss². The theoretically computed value of second moment for the rigid lattice is 17.99 gauss². A reasonable agreement exists in the theoretical and experimental value of second moment within the extent of experimental error. The lattice is rigid at the room temperature. The conformity between our experimental and the theoretical values of second moment justify the E.D.P. structure¹.

There exists no evidence of any sort of motion in the solid L-glutamic acid by electron density projection study or any other study at room temperature. This seems reasonable. The probability of molecular reorientation is least because of the relatively large size of the molecule and the way in which molecules link¹. The asymmetric carbon atom of the molecule is linked to the amino group, the (CH₂)₂COOH group and the carboxylic group. The rotation of (CH₂)₂COOH group is also not feasible about the C-C bond for the reasons of its mass and being attached to N atom which is surrounded by three oxygen atoms through at least two hydrogen atoms and the rotation of this group will involve the disturbance of extreme O atom separation of 2.54 Å. Similarly *r*-carboxylic group too, cannot rotate at a low temperature due to its structural features¹⁸. Again the amino group is linked to the other molecule by N-H...O bond and requires sufficient energy to reorient, depending upon the temperature¹⁹⁻²¹ of the transition. The evidence of amino group reorientation at high temperatures in solid amino acids are numerous^{15,22} and the problem of rotating interaction in connection with the fall of second moment value is dealt tacitly by Smith²³ and Kromhout *et al.*²⁴. Thus in our case the amino group rotation may take place at higher temperatures where the hindrance offered due to hydrogen bonding between the different substituent groups and also between different molecules is overcome. It is to be noticed that hyperfine structure of E.S.R. spectrum^{25,26} due to interaction of free electron spin with the hydrogen nuclei and the irreversible changes in the E.P.R. spectra^{27,28} at high temperatures and fine structure of P.M.R. derivative trace which is a consequence of the presence of the chemically non-equivalent protons; all are informative of the reorientation of the amino group at high temperatures. Again some of the probability of CH₂ group and the carboxylic group (not the *r* one) rotation seems to exist from microwave⁷ magneto-chemical²⁹ and U.V. absorption studies³⁰ but the structural features permit none of them at room temperature and a conclusive character of group reorientation can be ascribed only after the study at different temperatures and the respective second moment variations, which we could not study due to experimental difficulty.

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1. Sakutaro Hirokawa, *Acta Cryst.*, 1955, 8, 637.
2. Bernal, J. D., *Z. Kristallogr.*, 1931, 78, 363.
3. F. William, R. and M. John, T., *Appl. Spectros.*, 1971, 25(2), 175.
4. Balueva, G. R. and Terskov, I. A., *Sb. Dokl. Sib. Soves. Spektrosk. 3rd Krasnoyarsk USSR*, 1964, p. 140.
5. Patricia, N. Wancheck and Jeanette, G. Grassell, *Appl. Spectros.*, 1967, 21(5), 336.
6. McKimey, L. L., Uhing, E. H., Setzkorn, E. A. and Cowan, J. C., *J. Am. Chem. Soc.*, 1950, 72, 2599.
7. Walter, G. William, B. and Hoeards, S., *Proc. Natl. Acad. Sci. U.S.*, 1955, 41, 983.
8. Keith Harvey, D., Padden Frank, J. and Giannoni, G., *J. Mol. Bio.*, 1969, 43(3), 423.
9. Lakshminarayanan, A. V., Sasisekharan, V. and Ramachandran, G. N., *Conform. Biopolym. Pap. Int. Symp. (Mad.)*, 1967, 1, 62.
10. Brahm, J. and Spach, G., *Nature*, 1963, 200(4901), 72.
11. Akiyoshi, Wada, *J. Chem. Phys.*, 1955, 30, 328.
12. Agarwal, V. D., *NMR Study of Some Organic Compounds*, Ph.D. Thesis, Lucknow University, Lucknow, 1970.
13. Van Vleck, J. H., *Phys. Rev.*, 1948, 74, 1168.
14. Andrew, E. R. and Eades, R. G., *Proc. Phys. Soc.*, 1953, A66, 415.
15. Agarwal, S. C., Agarwal, P. and Gupta, R. C., *Journ. f. Prat. Chemie.*, Heft 3, 1973, S. 443.
16. Smith, G. W., *J. Chem. Phys.*, 1965, 42, 4299.
17. Andrew, E. R., *Phys. Rev.*, 1953, 91, 425.
18. Doyne, T. M., Hirokawa, S. and Watanabe, C., *Acta Cryst.*, 1954, 7, 652.
19. Gutowsky, H. S. and Pake, G. E., *J. Chem. Phys.*, 1950, 18, 162.
20. Kubo, R. and Tomita, K., *J. Phys. Soc. Japan*, 1954, 9, 888.
21. Waugh, J. S. and Fedin, E. I., *Fiz. tverdogo tela*, 1963, 4, 1633.
22. Banerjee, A. K., Mirza, P., Agarwal, P. A. and Gupta, R. C., *Ind. Jour. of Pure and Appl. Phys.*, 1974, 112, 768.
23. Smith, G. W., *J. Chem. Phys.*, 1965, 42, 4229.
24. Kromhout, R. A. and Moulton, W. G., *Ibid.*, 1955, 23, 1673.
25. Mc Cosmic Gen, G. and Gordy, W., *J. Phys. Chem.*, 1958, 62, 783.
26. Russel, C. Drew and Gordy, W., *Radiation Res.*, 1963, 18(4), 552.
27. Mochal Rion, A. I. and Kharitononkov, I. G., *Tr. Mosk. Obsochest. Ispyt. Priorotd. Bio.*, 1966, 16, 113.
28. Albert Van de Vorst, *C.R. Acad. Sci. (Paris)*, 1967, Ser. D, 264(8), 1100.
29. Roland Per Cean, *Compt. Rend.*, 1953, 236, 76.
30. Vinogradov, I. P., *Dodonova Nya. Opt. Spektrosk.*, 1971, 30(1), 27.