Report

INFRARED STUDIES OF C－H....O=C HYDROGEN BONDING IN POLYGLYCINE Ⅱ

Kiyoshi Kuroiwa.*

INTRODUCTION

The basic structure of polyglycine Ⅱ was determined by Crick and Rich (1955)on the basis of characteristic powder x－ray diffraction patterns found for this new form of polyglycine(Bamford.et al,1955). This structure con－sists of parallel polypeptide chains, each having a threefold screw axis, which are hydrogen－bonded via N－H…O＝C bonds to hexagonally－arrayed neighbor－ing chains. A small modification of this structure has been proposed recently (Ramachandran,Sasisekharan and Ramakrishnan,1966) in which it was suggested that a C－H....O＝C hydrogen bond can be formed in addition to the usual N－H…O＝C bond.

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The C(H)…O distance of 3.20A and the CH∧CO angle of 24^{o} found in this modified structure were taken as evidence for the possibility of forming such a bond in polyglycine Ⅱ, as had also been suggested for collagen (Ramachandran and Sasisekharan,1965). As yet, however, no independent evidence for the existence of such a bond has been provided. The purpose of this study is to determine if infrared spectroscopic evidence supports the presence of a C－H…O＝C hydrogen bond in polyglycine Ⅱ.

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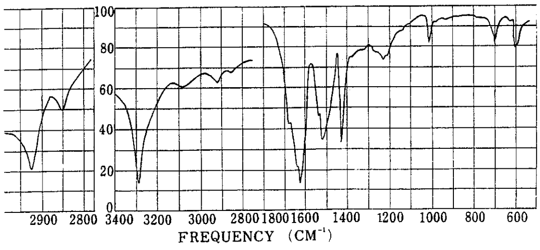
* Laboratory of Tokyo Customs,
Konancho,Minatoku,Tokyo.

As is well known (Pimentel and McClellan, 1960), the most prominent effect of hydrogen bonding on the vibrational spectrum is the lowering of the X—H stretching frequency. For example, the band at ～3300cm^{-1} in polyglycine represents the stretching vibration of the hydrogen—bonded N—H group, shifted down from the value of about 3450cm^{-1} for a free N—H group (Pimentel and McClellan, 1960). It would therefore be most appropriate to analyze the CH_2 stretching region of the spectrum for evidence of hydrogen bonding involving the C—H bond. Although there have been some experimental studies of the infrared spectrum of poly—glycine Ⅱ (Elliott and Malcolm, 1956; Suzuki,et al, 1966), no attention has been given to a detailed analysis of the CH_2 stretching vibrations. It should be noted that, although C—H hydrogen bonds are expected to be weaker than N—H hydrogen bonds (Pimentel and McClellan, 1960), crystal—lographic evidence does exist (Sutor, 1963) for short C(H)…O contacts in crystals (less than 3.3A), and therefore for some kind of attractive interaction in such a group.

EXPERIMENTAL AND RESULTS

The polyglycine used in this study was a polymer obtained from New England Nuclear Corp. Although its degree of polymerization is un—known, it is sufficiently high for our present purposes. Polyglycine I was prepared by casting a film or precipitating from dichloroacetic acid solution. Polyglycine Ⅱ was obtained by precipitation with water from a 1％ solution of the polymer in saturated aqueous lithium bromide. Specimens were deposited on AgCl plates, and spectra were obtained with a Perkin—Elmer 421 double—beam grating spectrophotometer. To study the CH_2 stretching vibrations in greater detail, ordinate and frequency scale expansions were used in the 2750—3050cm^{-1} region.

Typical spectra obtained for polyglycine I and polyglycine Ⅱ are shown in Figures 1 and 2 respectively. These are in good agreement with comparable spectra obtained by previous workers (Elliott and Malcolm, 1956; Bradbury



Infrared Studies of C—H···O=C Hydrogen Bonding in Polyglycine II

Fig.1 Infrared spectrum of polyglycine I

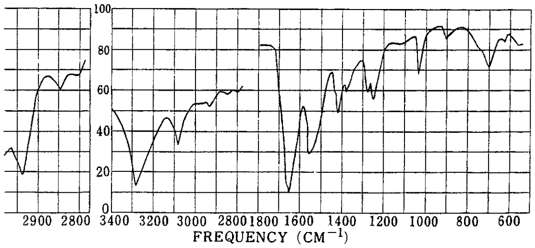


Fig.2 Infrared spectrum of polyglycine II,Sample 1

and Elliott, 1963 ; Suzuki, et al, 1966), although our spectra show several new details in the regions of special interest here. In particular, it is seen that while polyglycine I has two bands in the CH_2 stretching region, at 2850 cm^{-1} and 2923 cm^{-1}, in this region of the spectrum of polyglycine II there are clearly four bands, near 2800 cm^{-1},2848 cm^{-1},2935 cm^{-1} and 2980 cm^{-1}. Furthermore, we find that these four bands can be paired off on the basis of some variability in relative intensities from one sample to another : the 2800cm^{-1} and 2980 cm^{-1} bands increase or decrease together with respect to the pair at 2848 cm^{-1} and 2935 cm^{-1}. In one case the band at 2800 cm^{-1} was more intense than that at 2848 cm^{-1}.(see Fig.3) It should also be

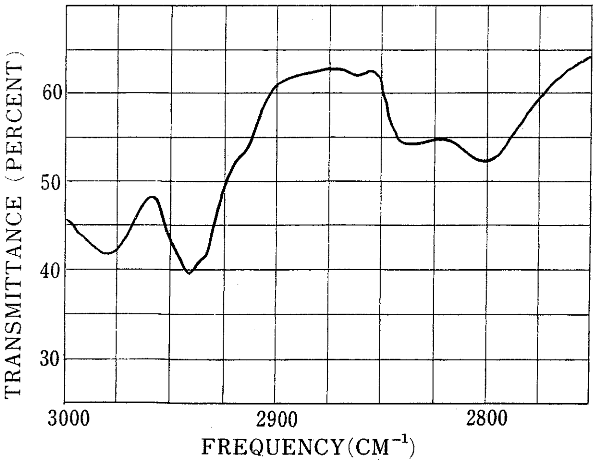


Fig.3 Infrared spectrum of polyglycine II, Sample 2

noted that polyglycine II has a shoulder at 1432 cm^{-1}, on the side of the strong band at cm^{-1}, Although the frequency of this shoulder coincides with that of the characteristic band of polyglycine I, we do not believe that this indicates the presence of polyglycine I in this specimen, since the remainder of the spectrum shows no evidence for such an admixture (cf., for example, the absence of a band at 1017 cm^{-1}, characteristic of polyglycine I, and the presence of a sharp band at 1028 cm^{-1}, characteristic of polyglycine Ⅱ). The 1432 cm^{-1}, shoulder of polyglycine Ⅱ is believed to be associated with the spectrum of this structural form.

DISCUSSION

A rigorous analysis of the effects of hydrogen bonding on the spectrum in the CH_2 stretching region would require a normal coordinate calculation for polyglycine, which has not as yet been achieved. It is possible, however, to undertake a meaningful inquiry into possible changes due to hydrogen bonding because the vibrational modes of the CH_2 group are almost com— pletely separable normal coordinates. This is indicated by the values of the two CH_2 stretching frequencies in polyglycine I, at 2850cm^{-1} and 2923cm^{-1} , which are within a few cm^{-1} of the values found in many vinyl polymers (Krimm,1960), where it is known that the modes are essentially 100% CH_2 stretching (cf. Snyder and Schachtschneider, 1964). In the polypeptide chain the peptide group vibrations do not interact with the CH_2 vibrations, and thus the peptide groups effectively act to isolate CH_2 groups from each other. It is most likely that the same is true in the polyglycine Ⅱ structure. It is thus possible to examine the CH_2 stretching bands with the knowledge that we are looking at modes that are essentially unperturbed by changes in intramolecular conformation.

A prominent feature in the spectrum of polyglycine Ⅱ is the appearance in the CH_2 stretching region of two new bands at 2800cm^{-1} and 2980cm^{-1}. Are these bands to be associated with CH_2 stretching vibrations？ This would appear to be the case on the basis of two arguments. First, these bands disappear from the spectrum when CD_2 is substituted for CH_2, just as do the bands at 2848cm^{-1} and 2935cm^{-1}, but are unaffected by substi -

tution of N—D for N—H or 15$_N$ for 14$_N$ in the polymer (Suzuki, et al,1966). Second, the intensities of these bands are comparable to the intensities of the CH$_2$ stretching fundamentals at 2848cm^{-1} and 2935cm^{-1} , making it difficult to associate them with overtone or combination bands. Nor is it evident that new strong overtones or combinations are expected for polyglycine Ⅱ over what would occur in the spectrum of polyglycine I. We conclude therefore that the bands at 2800cm^{-1} and 2980cm^{-1} in the spectrum of polyglycine Ⅱ are to be correlated with CH$_2$ stretching modes.

The next question to be considered is why the spectrum of polyglycine Ⅱ shows four CH$_2$ stretching vibrations while that of polyglycine I has only two. In polyglycine I only two CH$_2$ stretching modes are expected to be infrared active, even though the repeat unit along the chain contains two CH$_2$ groups, because of the two—fold screw axis of symmetry parallel to the chain axis. The active modes are those chain vibrations which are antisymmetric to this two—fold axis, the 2850cm^{-1} band corresponding to the CH$_2$ symmetric stretching mode and the 2923cm^{-1} band to the CH$_2$ antisymmetric stretching mode (Krimm, 1960). If we consider the polyglycine Ⅱ chain, with its three—fold screw axis of symmetry (Crick and Rich, 1955), then we would expect a splitting of the CH$_2$ group modes into A and E species chain vibrations (Krimm, 1960). However, it is soon evident that this splitting, arising from an intrachain interaction, is most unlikely to account for the four observed bands of polyglycine Ⅱ. In the three—fold helical structure of polypropylene, for example, such splittings are of the order of a couple cm^{-1}(Snyder and Schachtschneider, 1964), and we would not anticipate larger splittings in polyglycine Ⅱ where the CH$_2$ groups are even more isolated from one another. The～40 cm^{-1} splitting in the polyglycine Ⅱ spectrum which would have to be assigned to such an effect (e,g.,the 2800cm^{-1} and 2848cm^{-1} bands being correlated with the two helix-mode CH$_2$ symmetric stretching vibrations) is thus too large to be attributed to intrachain interactions. Furthermore, the relative intensity changes in these bnads which are observed between samples precludes their having such a similar origin. We conclude therefore that the appearance of additional bnads in the spectrum is a result of new interchain environmental effects.

It is pertinent to note that in the structures proposed thus far for polyglycine Ⅱ (Crick and Rich, 1955；Ramachandran, Sasisekharan and Ramakrishnan, 1966) all CH_2 groups are in equivalent environments, and thus only two CH_2 stretching frequencies could be expected. This arises from the fact that in these structures all polypeptide chains are directed parallel to each other. It has been pointed out, however, that independent arguments require that there be antiparallel chains in polyglycine Ⅱ (Krimm,1966). There is therefore the possibility of having two kinds of environments for a CH_2 group, namely that between parallel chains and that between antiparallel chains. The former environment permits the formation of a $C-H\cdots O=C$ hydrogen bond (Ramachandran, Sasisekharan and Ramakrishnan, 1966)； it has been found that in the latter case only a very much weaker bond (if any at all) can form (Ramachandran, 1966). We thus have a reasonable explanation for the four observed bands of polyglycine Ⅱ：the 2848cm^{-1} and 2935cm^{-1} bands are due to the (essentially) unbonded CH_2 groups (since their frequencies are so close to those of polyglycine I), whereas the 2800 cm^{-1} and 2980cm^{-1} bands arise from bonded CH_2 groups. The small intensity differences between these pairs of bands would then be accounted for by minor variations in the ratio of parallel to antiparallel chain environments.

In order to get a better understanding of the origin of the shifts represented by the 2800cm^{-1} and 2980cm^{-1} bands, a normal vibration analysis was performed on the model four－atom system $CH_2\cdots O$. This calculation should be a meaningful one since the modes of the CH_2 group are essentially completely separable from the rest of the polypeptide chain. Standard values were used for the C－H bond length and the H－C－H angle. Force constants were taken from a valence force field derived for hydrocarbons (Snyder and Schachtschneider, 1965), and were modified slightly to give reasonable agreement with the frequencies of polyglycine I. Thus, for $k(C-H)=4.580$ ($x10^5$ dynes/㎝) and $K(H-C-H)=0.660$ we find frequencies of 2928cm^{-1}, 2856cm^{-1} ,and 1433cm^{-1} for the CH_2 antisymmetric stretching, symmet－

ric stretching, and bending frequencies, respectively. As a model for a hydrogen-bonded $CH_2 \cdots O$ group, the oxygen atom was placed in the CH_2 plane at a distance of 3.20A from the carbon atom and such that the $CH \wedge CO$ angle was 24^o, corresponding closely to the proposed modified structure of polyglycine Ⅱ (Ramachandran, Sasisekharan and Ramakrishnan, 1966). (Placing the O atom out of the CH_2 plane by amounts comparable to those in the modified structure produced no significant differences in the frequencies.) Various values were taken for $k(H \cdots O)$, the hydrogen bond force constant, but the results were relatively insensitive to this constant over a reasonable range. The calculations brought out two main points. First, only by increasing one C—H stretching constant and simultaneously decreasing the other could the two original CH_2 stretching frequencies of polyglycine I be further separated. Decreasing only one stretching constant caused both frequencies to decrease. Second, the bending frequency depended almost entirely on the bending force constant alone. Changing the stretching force constants by 6%, viz., $k(C-H_o)=4.305$ and $k(C-H)=4.855$, and decreasing the bending constant by 3%, viz., $k(H-C-H)=0.644$, gave, for $k(H \cdots O)=0.02$, predicted frequencies of $2985cm^{-1}$, $2801cm^{-1}$, and $l416cm^{-1}$, in reasonable agreement with the observed values for polyglycine Ⅱ ($C-H_o$ refers to the carbon-hydrogen bond whose hydrogen participates in bonding to the oxygen atom). We are assuming here that the observed band at $1418cm^{-1}$ is correlated with the bonded CH_2 groups, the unshifted shoulder at $l432cm^{-1}$ being associated with the nonbonded groups.

The bonding of a hydrogen atom of a CH_2 group to an oxygen atom would be expected to be accompanied by a decrease in the stretching force constant of the C—H bond. The decrease of about 6% indicated by the above model calculation seems reasonable for the weak bond involved. A simple model calculation of the change in force constant of the N—H bond when the hydrogen atom bonds to a $O=C$ group indicates a decrease of about 10%, showing this to be (as expected) a stronger hydrogen bond. The increase in the force constant of the other C—H bond which is required to give agreement with the observed frequencies may seem unusual at first glance, but on second thought it appears quite reasonable. If the electron distribution about the alpha carbon atom becomes altered in a manner to weaken one C—H bond, it is expected that the electron distribution in the other bonds will change in such a way as to strengthen these bonds (Coulson,1952). In fact, the stretch—stretch interaction constant in the valence force field for hydrocarbons (Snyder and Schachtschneider, 1965) is of such a sign as to indicate that the stretching of one C—H bond makes it harder to stretch the other, in qualitative agreement with the above reasoning. From this point of view the changes which are found to be necessary in both stretching constants of the CH_2 group furnish positive evidence for the involvement of one hydrogen atom in a hydrogen—bonding type of interaction. It is more difficult to predict the change that might occur in the bending force constant, but the value arrived at above does not seem unreasonable.

CONCLUSION

An analysis of the infrared spectroscopic evidence on polyglycine Ⅱ is thus seen to support the suggestion that a C—H…O=C hydrogen bond is present in this structural form (Ramachandran, Sasisekharan, and Ramakrishnan, 1966). It also confirms the proposal that polyglycine Ⅱ must contain antiparallel chains (Krimm, 1966).

The consequences of both of these points are significant to the principles of protein structure. The first suggests that closer contacts between Cα—H and O=C may be allowed than are indicated by steric considerations alone (Ramakrishnan and Ramachandran, 1965；Leach, Nemethy, and Scheraga, 1966). This may be particularly true if the Cα atom is part of a glycine residue, but interactions involving other residues may also be possible. Since the formation of a C—H…O=C hydrogen bond leads to an additional lowering of the energy, this might explain why the polyglycine Ⅱ conformation is favored over an alpha—helix, in which such a bond is not possible. The second point is relevant to the structure of collagen, which is closely related to that of polyglycine Ⅱ. The structures proposed heretofore for collagen (Rich and Crick, 1961； Ramachandran and Sasisekharan, 1965, and earlier papers) have been based on parallel chains. The demonstration that antiparallel chains exist in polyglycine Ⅱ suggests that the feasibility of building three－stranded collagen helices with one strand antiparallel to the other two should be examined.

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Summary

Infrared studies of polyglycine Ⅱ have been undertaken in order to test a proposal that C—H...O=C hydrogen bonds are present in this structure. An analysis of the CH_2 stretching region indicates that two new bands found in the spectrum of polyglycine Ⅱ, which are not present in polyglycine I,can very reasonably be assigned to hydrogen bonded CH_2 groups. These results confirm a previous suggestion that polyglycine Ⅱ contains antiparallel chains.