Intramolecular Rotation and the Structure of High Polymers. II. Further Studies on the Structure of Polypeptide Chain

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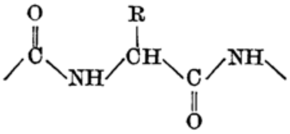
I. Proposed Configurations of a Polypeptide Chain

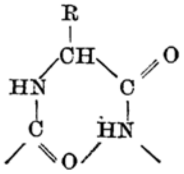
Based on the energy consideration of intramolecular rotation about a single bond as axis, two of us proposed two unit structures of a polypeptide chain,(1)



$$\diagup \overset{\overset{O}{\|}}{C} \diagdown NH \diagup \overset{\overset{R}{|}}{C}H \diagdown \underset{\underset{O}{\|}}{C} \diagup NH \diagdown$$

(E-form)

and



(B-form).

Taking into account that all amino acid residues of the polypeptide chain are in L-forms, we can construct various configurations of a polypeptide chain shown in Fig. 1, of which EEE···· corresponds to the extended form presented by Meyer and Mark in 1928,(2) BBB···· to a possible configuration of α-keratin presented by us in 1947,(1) and independently by Ambrose *et al.* in 1949,(3) and BBEBBE···· to another possible configuration of α-keratin presented by us in 1948.(1) These figures represent

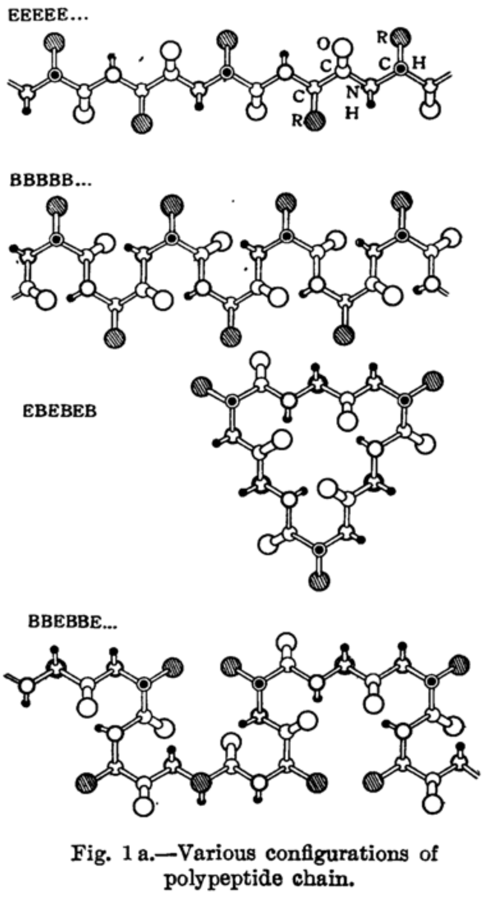


Fig. 1 a.—Various configurations of polypeptide chain.

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(1) Shimanouchi and Mizushima, *Kagaku*, **17**, 24 and 52 (1947); *Bull. Chem. Soc. Japan*, **21**, 1 (1948); *Chem. Abst.*, **43**, 8843 (1949).

(2) Meyer and Mark, *Ber.*, **61**, 1932 (1928).

(3) Ambrose, Elliot and Temple, *Nature*, **163**, 859 (1949).

the projections of the molecular configurations on a plane perpendicular to one of the carbon bonds, where for the sake of simplicity all the bond angles of carbon and nitrogen are assumed to be tetrahedral, all the bond lengths of C-C and C-N to be equal and all the intramolecular rotational states about the C-C and C-N bonds as axes to be either the *trans* ($\theta=0°$) or the *gauche* ($\theta=120°$).

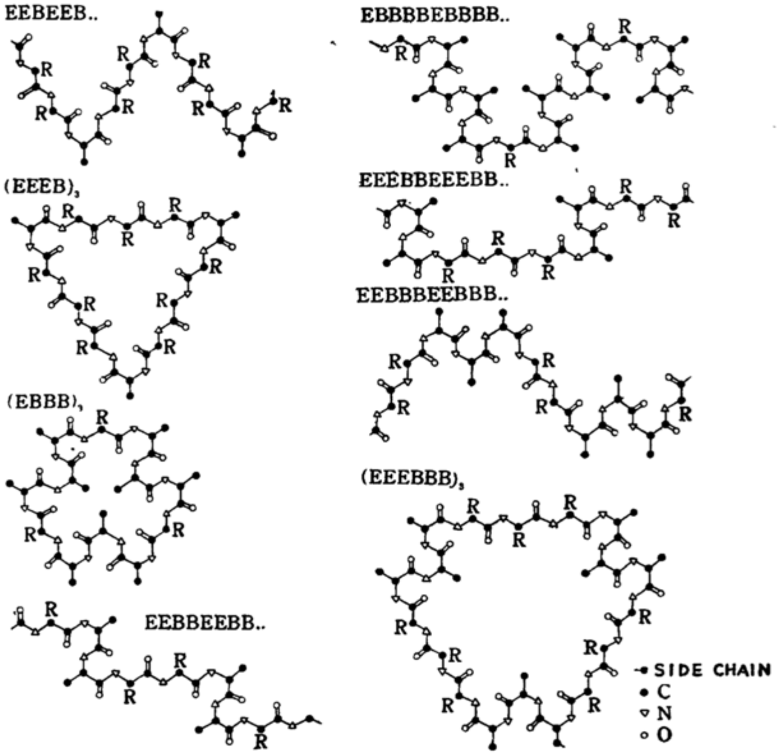

Fig. 1 b.—Various configurations of polypeptide chain. (Side chains R perpendicular to the plane of the paper are not shown in the figures.)

In an actual case the bond angles and the bond lengths would be in some measure different from these ideal values and, moreover, the intramolecular rotational state would easily be changed to a certain extent under the influence of the neighboring molecules. If, for example, we assume the bond lengths and bond angles of polypeptide chain to have the values equal to those found for glycylglycine:[4]

$$\begin{array}{ll} C_\alpha\text{—}N = 1.48\ \text{Å}. & \angle C\text{—}C_\alpha\text{—}N = 110° \\ N\text{—}C = 1.29\ \text{Å}. & \angle C_\alpha\text{—}N\text{—}C = 122° \\ C\text{—}C_\alpha = 1.53\ \text{Å}. & \angle N\text{—}C\text{—}O = 125° \\ C\text{—}O = 1.23\ \text{Å}. & \angle N\text{—}C\text{—}C_\alpha = 114° \end{array}$$

and the intramolecular rotational angles to have the following values:

$$\theta(\text{NH-CO-C}_\alpha\text{-NH}) = 140°$$
$$\theta(\text{CO-C}_\alpha\text{-NH-CO}) = 110°,$$

the period along the chain is calculated as 5.14 Å. in agreement with the observed value 5.15 Å. and the N-H····O distance in the B form as 2.52 Å. which is also reasonable.

As stated in our previous paper and as pointed out by Astbury, the presence of a twofold screw axis seems at first sight to be inconsistent with the fact that the 5.1 Å. meridional reflexion found in α-keratin is strong and well defined.(5) However, according to the experimental result obtained by Bamford, Hanby and Happey, a kind of synthesized polypeptides gives an α-keratin diagram in which the 5.28 Å. polar arc is weak and diffuse.(6) In view of this fact it is not unreasonale to propose the BBB···· configuration

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(4) Hughes and Moore, *J. Am. Chem. Soc.*, **71**, 2618 (1949).

(5) Astbury, *Nature*, **164**, 439 (1949).

(6) Bamford, Hanby and Happey, *Nature*, **164**, 138 (1949).

as one of the possible structure of α-keratin

In the case of BBEBBE···· configuration of α-keratin the reflexion data can be explained more simply. If the mechanism of stretching of keratin fiber is explained in terms of the transformation of BBEBBE···· into EEE····, the amount of elongation is calculated as about 90%, while the side chain spacing remains unchanged. (See Fig.2.)

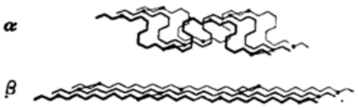
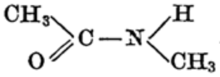


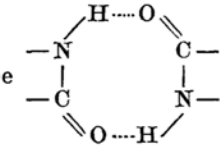
Fig. 2.—Natural α-keratin considered to be in BBEBBE······ configuration and the β-keratin in EEE······ configuration.

II. Experimental Evidence in Support of Our View

Since our previous paper(1) was published, the experimental evidence in support of our view has been accumulated in our laboratory as follows:

(1) Our experimental results of the Raman effect, infra-red absorption, ultra-violet absorption and dipole moment on N-methyl acetamide have shown that the molecule is in the *trans* configuration  .(7) This is in conformity with our view that all the peptide bonds contained in the main chain of polypeptide are in the *trans* configuration (see Fig. 1).

(2) When involved in hydrogen bonding, N-methyl acetamide shows two absorption peaks at 3.0 μ and at 3.2 μ arising from the N-H vibration.(7,8) N-ethyl acetamide and N-cyclohexyl acetamide, whose peptide bonds are shown to be in the *trans* configuration, also show two absorption peaks at the same wave lengths.(9) On the other hand δ-valerolactam, ε-caprolactam, diketopiperazine, etc. whose peptide bonds are considered to be nearly in the *cis* configuration, show two absorption peaks at 3.1 μ and at 3.2 μ.(8) This difference in wave length of the hydrogen-bonded N-H bands is attributable to the difference in the position of H-atom relative to other atoms and we can tell whether the peptide bond is in the *trans* configuration or in the *cis* configration according as the absorption peaks of the hydrogen-bonded N-H bands appear at 3.0 μ and 3.2 μ or at 3.1 μ and 3.2 μ.

Since in egg albumin, horse serum albumin, etc. measured in our laboratory(8) and other proteins measured by Buswell *et al.*(10) only the 3.0 μ and 3.2 μ absorption peaks are observed except in salmin which shows the 3.1 μ peak arising from the side chain, the main chain of polypeptide can be considered to contain peptide bonds always in the *trans* configuration. Therefore, the intermolecular hydrogen bond of the type  would not be formed between two main chains of polypeptide.

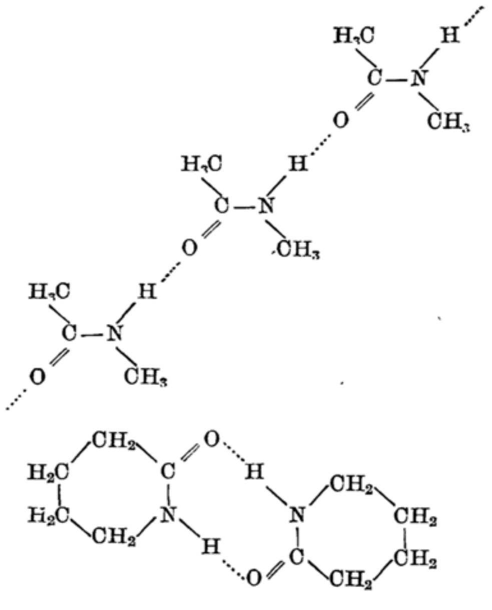


Fig. 3.—Configurations of associated molecules of N-methylacetamide and δ-valerolactam.

(3) It has been shown by our infra-red absorption measurement that all the compounds except the first two shown in Table 1 can take the B form,(11,12) e.g. acetylglycine N-methyl amide can have a configuration:

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(7) Mizushima, Shimanouchi, Nagakura, Kuratani, Tsuboi, Baba and Fujioka, *J. Am. Chem. Soc.*, **72**, 3490 (1950)

(8) Tsuboi, *Bull. Chem. Soc. Japan*, **22**, 215 and 255 (1949).

(9) Buswell, Rodebush and Roy, *J. Am. Chem. Soc.*, **60**, 2444 (1938).

(10) Buswell, Krebs and Rodebush, *J. Phys. Chem.*, **44**, 1126 (1940).

(11) Mizushima, Shimanouchi, Tsuboi, Sugita and Kato, *Nature*, **164**, 918 (1949).

(12) Mizushima, Shimanouchi, Tsuboi, Sugita, Kato and Kondo, to be published shortly.

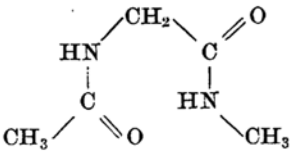
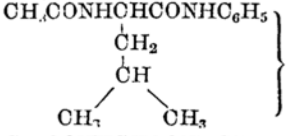
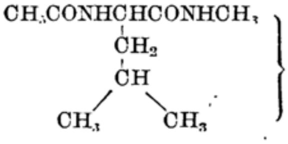


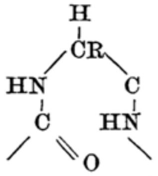
Table 1

Absorption Peaks Arising from N-H Vibrations

Compound	Absorption peaks arising from the vibration of N-H group	
	in the free state, μ	involved in intramolecular hydrogen bonding, μ
$CH_3CONHC_6H_5$	2.90	2.97 (intermolecular)
$CH_3CONHCH_3$	2.88	2.97 (intermolecular)
$CH_3CONHCH_2CONHC_6H_5$	2.90	3.00
$CH_3CONHCH_2CONHCH_3$	2.90	2.98
 $CH_3CONHCH(CH_2CH(CH_3)_2)CONHC_6H_5$	2.90	3.00
 $CH_3CONHCH(CH_2CH(CH_3)_2)CONHCH_3$	2.90	2.98

In very dilute solutions these substances show two absorption peaks as shown in Table 1, of which the 2.9 μ peak can be assigned to the vibration of N-H group in the free state and the 3.0 μ peak to that involved in the *intramolecular* hydrogen bonding. The soundness of the assignment of the 3.0 μ peak to the *intramolecular* hydrogen bonding and not to the *intermolecular* hydrogen bonding can be seen from the fact that the intensity ratio of the 3.0 μ to the 2.9 μ band is independent of the concentration in dilute solutions of acetylglycine N-methyl amide while in solutions of acetanilide and N-methyl acetamide for which only the *intermolecular* hydrogen bonds are formed, the 3.0 μ band becomes weaker with increasing dilution until it disappears practically. (See Table 1.) Thus there is no doubt that molecules of the substances shown in Table 1 can take the B form. This would also be an evidence for the existence of the B form for certain amino acid residues contained in the polypeptide chain.

III. Configurations Presented by Other Investigators

Let us next discuss on the molecular configurations presented recently by other investigators. The configuration of α-keratin proposed by Huggins has six membered rings similar to that of our configuration BBB····, but it is different from ours with respect to the position of the side chain.(13) In the unit structure of his model  H and R are interchanged when compared with our B form. According to the energy consideration of intramolecular rotation his model containing more *gauche* forms than ours would be less stable than ours. This would be seen, for example, from the molecular configuration of monochlorocyclohexane recently determined by Kojima.(14) Of the two conceivable configurations (equatorial and polar) of this molecule only the former has been observed in the Raman effect. This stable equatorial form corresponds in the present case to our B form and the unobserved polar form containing more *gauche* configurations than the equatorial corresponds to that presented by Huggins. The shape of pyranose rings determined by Reeves would provide us with another evidence in support of this view.(15)

Based on the measurement with polarized infra-red radiation Ambrose *et al.* proposed independently a molecular model of α-keratin identical with our model BBB····.(3) This would be another evidence in support of our view. However, their experimental result can also be explained with our model BBEBBE····. More detailed discussion on the observed dichroic ratio and the real molecular configuration stated in section I would be desirable.

Darmond and Sutherland measured the N-H frequencies of compounds containing peptide bonds, but arrived at a conclusion different from ours.(16) They consider that the 3.0∼3.1 μ band arises from a configuration different from that to which they assigned the 3.2 μ band. According to our measurement, however, both of the 3.0 μ (or 3.1 μ) and the 3.2 μ bands arise from a single configuration in the case of N-methyl acetamide, acetanilide, and diketopiperazine in the solid state and δ-valerolactam and ε-caprolactam in solutions.(7,8) (The proof for the single configuration for these substances is given by the X-ray diffraction, Raman effect,

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(13) Huggins, *Chem. Rev.*, **32**, 195 (1943).

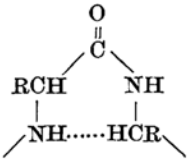
(14) Kojima, Private Communication.

(15) Reeves, *J. Am. Chem. Soc.*, **72**, 1499 (1950).

(16) Darmond and Sutherland, *Nature*, **164**, 440 (1949).

infra-red absorption and dielectric measurement). For the dimer of carboxylic acids it is also known that more than one O—H vibration arise from a single configuration.(17) Therefore, the appearance of more than one N—H stretching frequencies cannot be a proof for the existence of more than one type of hydrogen bond. Thus the objection of Darmond and Sutherland to the structure of α-keratin proposed by us and by Ambrose *et al.* cannot be approved by our infra-red measurement.

Quite recently Kanda has presented another configuration of α-keratin, the unit structure of which has the following form:(18)



His discussion is based on the consideration of interatomic distances, but not of the energy relation of the intramolecular rotation. According to the latter point of view, such a configuration is not acceptable and moreover, the hydrogen bond CH····N contained in his configuration is supported by no experimental evidence. In the case of N≡C—H there is evidently a hydrogen bond of the type N≡CH····N as pointed out by Kanda, but this is due to the abnormal property of the CH group of N≡C—H given rise to by the resonance with a structure

$$N^- = C^+ - H.$$

On the contrary CH groups contained in the main chain of polypeptide —CO—CHR—NH— have normal property and the nitrogen atoms next to carbon atoms have rather acidic property. Therefore, a resonance structure such as $>C^+$—H cannot be considered and the nitrogen atoms are not qualified to be a proton accepter. It has never been reported that the hydrogen bond of the type C—H····N is formed between such a CH group and a nitrogen atom.

Kanda's objection to our configuration of α-keratin is based on the consideration of the interatomic distances for the ideal model shown in Fig. 1. However, as stated in Section I, a reasonable change of bond distances, bond angles and intramolecular rotational states from the ideal values reconciles our configuration with the experimental data.

In conclusion we wish to thank Drs. N. Tanaka and K. Kuratani for their discussions. This work was aided by a grant from the Ministry of Education.

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(17) Buswell, Rodebush and Roy, *J. Am. Chem. Soc.*, **60**, 2239 (1938); Wall and Claussen, *J. Am. Chem. Soc.* **61**, 2812 (1939).

(18) Kanda, *Report of the Annual Meeting of the Chemical Society of Japan*, (1950).