生体分子構造解析学特論

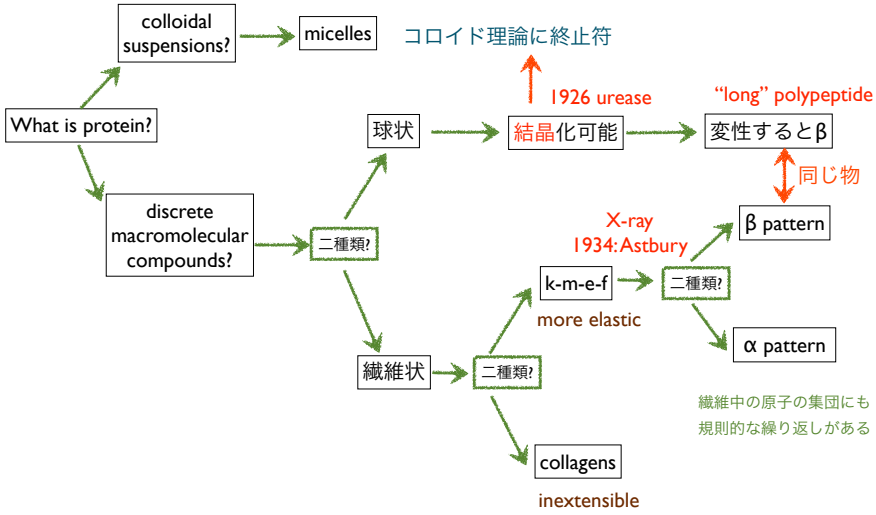
シンクロトロン光研究センター
渡邉 信久

第2回

講義スケジュール

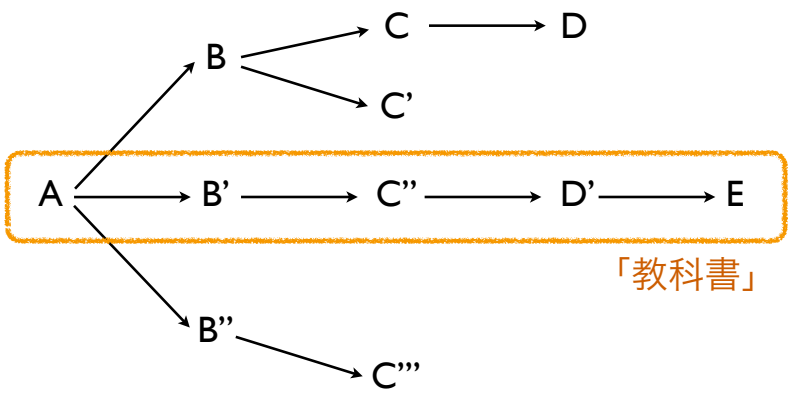
- 1：混沌の時代から繊維写真の時代
- 2：サイクロール説
- 3：二次構造の解明
- 4：DNAの構造
- 5：結晶構造解析法の発展
- 6：高分解能構造解析の始まり

「歴史」が辿った道筋



球状タンパク質の構造の探求

「歴史」が辿る道筋



誤った解釈

「正解」を知っている現在の目から見ると…

- Wrinch: cyclol
- Perutz: α-helix
- Pauling: triple DNA

key paper

Wrinch は Astbury の
繊維写真の解釈を
どう誤って解釈して
しまったのか

The Pattern of Proteins
By Dr. D. M. Wrinch, Mathematical Institute, Oxford
Nature (1936) 137, 411-412

The Cyclol Theory and the 'Globular' Proteins*
By Dr. D. M. Wrinch
Nature (1937) 139, 972-973

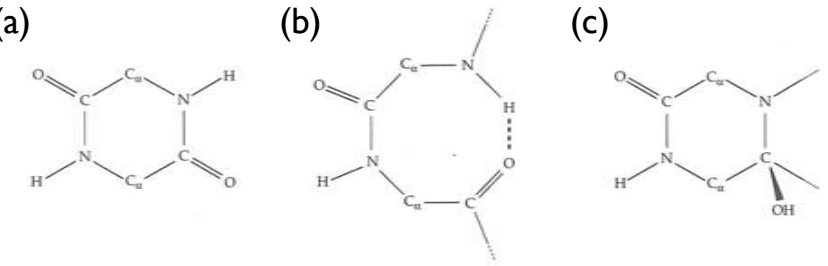
化学結合論による論考

The Structure of Proteins
By Linus Pauling and Carl Niemann
JACS (1939) 61, 1860-1867

Wrinchの反論

The Geometrical Attack on Protein Structure
By Dorothy M. Wrinch
JACS (1941) 63, 330-333

(a) (b) (c)

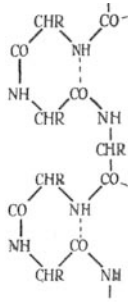


diketopiperazine　H-bonded β bend　cyclol ring

WT Astbury, *Phil. Trans. Roy. Soc. London* (1934) A232, 333-394

The β-form is thus represented by fully-extended peptide chains in which each amino-acid residue takes up, on the average, a length along the fibre-axis of 3·4 A, while the α-form is represented by a series of pseudo-diketopiperazine rings which follow each other according to a pattern of length 5·1 A. The unfolding of the rings is clearly accompanied by an elongation of 100%, and the suggested pattern offers an explanation of both the characteristic meridian reflection of the α-form (5·1 A) and of the decrease of resistance of the β-form, as compared with the α-form, to the action of reagents such as steam, etc.

p.344

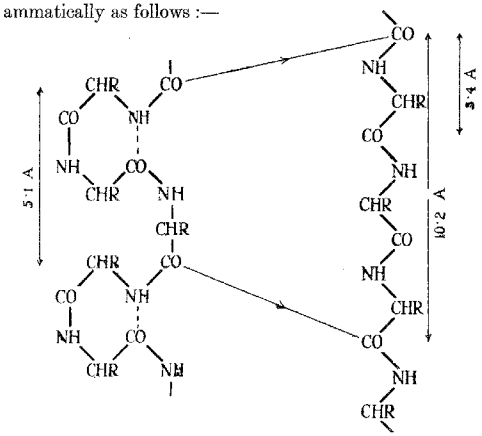


pseudo-diketopiperazine rings

Astburyのモデル

α pattern　　β pattern

ammatically as follows :—



5.1Å

10.2Å

key paper

DM Wrinch, *Nature* (1936) 137, 411-412

MARCH 7, 1936　　NATURE　　411

The Pattern of Proteins

By Dr. D. M. Wrinch, Mathematical Institute, Oxford

Wrinch の cyclol

p.411

ANY theory as to the structure of the molecule of simple native protein must take account of a number of facts, including the following :

(1) The molecules are largely, if not entirely, made up of amino acid residues. They contain —NH—CO linkages, but in general few —NH_2 groups not belonging to side chains, and in some cases possibly none.

(2) There is a general uniformity among proteins of widely different chemical constitution which suggests a simple general plan in the arrangement of the amino acid residues, characteristic of proteins in general. Protein crystals possess high, general trigonal, symmetry.

(3) Many native proteins are 'globular' in form.

(4) A number of proteins[1] of widely different chemical constitution, though isodisperse in solution for a certain range of values of *p*H, split up into molecules of submultiple molecular weights in a sufficiently alkaline medium.

(1) 側鎖に由来しないフリーのアミノ基はほとんどない.

(2) 蛋白質は3回対称を持つ.

The facts cited suggest that native protein may contain closed, as opposed to open, polypeptides, that the polypeptides, open or closed, are in a folded

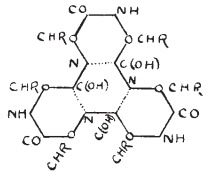


FIG. 1. The 'cyclol 6' molecule.

state, and that the type of folding must be such as to imply the possibility of regular and orderly arrangements of hundreds of residues.

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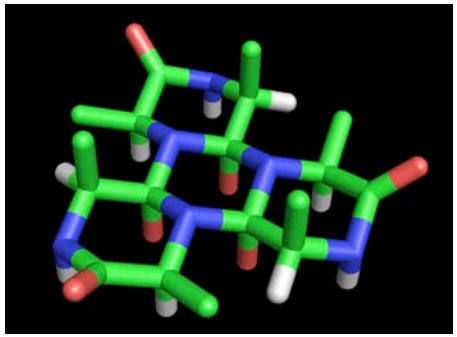
The alanine cyclol-6 molecule



http://en.wikipedia.org/wiki/Cyclol

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mathematics...

An examination of the geometrical nature of polypeptide chains shows that, *since all amino acids known to occur in proteins are α-derivatives*, they may be folded in hexagonal arrays. Closed polypeptide chains consisting of 2, 6, 18, 42, 66, 90, 114, 138, 162 . . . $(18 + 24n)$ . . . residues form a series with threefold central symmetry. A companion series consisting of 10, 26, 42, 58, 74, 90, 106, 122 . . . $(10 + 16n)$ . . . residues have twofold central symmetry. There is also a series with sixfold central symmetry : others with no central symmetry. Open polypeptides can also be hexagonally folded. The number of free —NH_2 groups, in so far as these indicate an open polypeptide, can be made as small as we please, even zero if we so desire. The hexagonal folding of polypeptide chains, open or closed, evidently allows the construction of molecules containing even hundreds of amino acid residues in orderly array,

By using the transformation* suggested by Frank in 1933 at a lecture given by W. T. Astbury to the Oxford Junior Scientific Society,

$$\diagdown C=O \quad H\text{–}N \quad \text{to} \quad \diagdown C(OH)\text{–}N\diagup$$

which has already proved useful in the structure of α-keratin[3], the situation is at once cleared up and we obtain (Fig. 1) the molecule 'cyclol 6' (the closed

already proved?

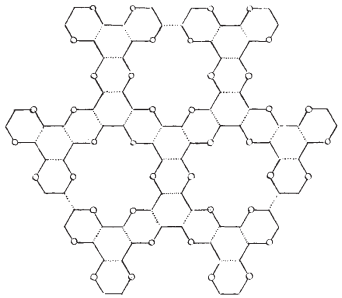

FIG. 2. A 'cyclol 42' molecule.

polypeptide with six residues), 'cyclol 18', 'cyclol 42' (Fig. 2) and so on, and similarly open 'cyclised' polypeptides (Fig. 3).

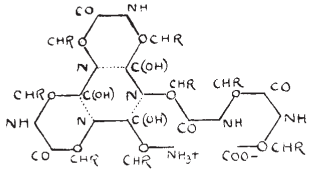

FIG. 3.

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key paper

DM Wrinch, *Nature* (1937) 139, 972-973

The Cyclol Theory and the 'Globular' Proteins*

By Dr. D. M. Wrinch

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p.972

A NUMBER of facts relating to proteins[1] suggest that the polypeptides in native proteins are in a folded state[2,3]. The type of folding must be such as to imply the possibility of the regular and orderly arrangement of hundreds of amino acid residues, which to some extent at least is independent of the particular residues in question.

At present two types of folding have been suggested, the cyclol type[3,4] and the hydrogen bond type[5]. The search for other types of folding is being continued. So far, it has not proved possible to discard either theory on the grounds that the type of link postulated is out of the question. It is, therefore, very desirable to test these theories by checking their implications against known facts. Accordingly it is now considered whether the cyclol theory can stand the test of the body of facts relating to the 'globular' proteins, established by Svedberg and his collaborators[6].

In previous communications the cyclols have been considered only in the case when all the median hexagons lie in one common plane. With this limitation there has, of course, been no question of building a closed (that is, a space-enclosing) cyclol. To do so, it is necessary to investigate the conditions under which a cyclol fabric can bend about a line. Evidently it is permissible for two abutting median hexagons to lie on different planes, if the angle between the planes is the tetrahedral angle δ. Thus a cyclol

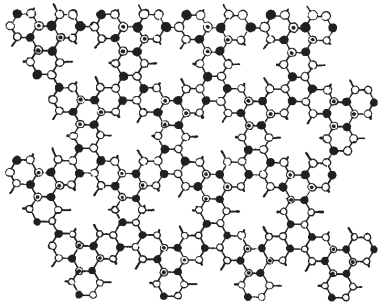

Fig. 1.

THE CYCLOL PATTERN. THE MEDIAN PLANE OF THE LAMINA IS THE PLANE OF THE PAPER. THE LAMINA HAS ITS 'FRONT' SURFACE ABOVE AND ITS 'BACK' SURFACE BELOW THE PAPER.

● = N.
○ = C(OH), PEPTIDE HYDROXYL UPWARDS.
⊙ = C(OH), PEPTIDE HYDROXYL DOWNWARDS.
○— = CHR, DIRECTION OF SIDE CHAIN INITIALLY OUTWARDS.
○- = CHR, DIRECTION OF SIDE CHAIN INITIALLY UPWARDS.

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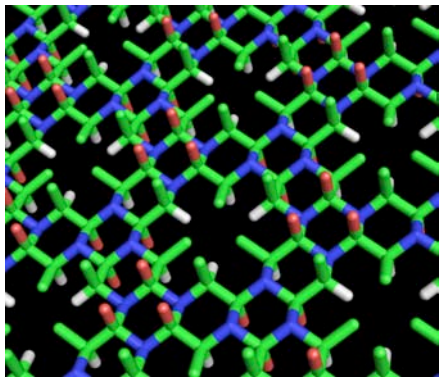
Stick model of the alanine cyclol fabric



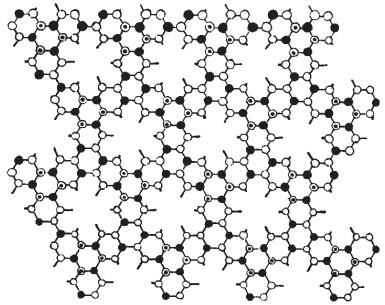


Fig. 1.

THE CYCLOL PATTERN. THE MEDIAN PLANE OF THE LAMINA IS THE PLANE OF THE PAPER. THE LAMINA HAS ITS 'FRONT' SURFACE ABOVE AND ITS 'BACK' SURFACE BELOW THE PAPER.

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○- = CHR, DIRECTION OF SIDE CHAIN INITIALLY UPWARDS.

http://en.wikipedia.org/wiki/Cyclol

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THE CLOSED CYCLOLS

To solve this problem, all the polyhedra in which some at least of the dihedral angles are equal to the tetrahedral angle will be considered in turn. As a first step it is remarked that among the regular and semi-regular polyhedra[8], only four satisfy the conditions. These are the truncated tetrahedron, the octahedron, the truncated octahedron and the skew triangular prism. On this occasion, as an example of this method of building megamolecules, attention is directed to the truncated tetrahedron, on which it has proved possible to draw closed cyclol networks. These networks form a linear series $C_1, C_2, \ldots C_n, \ldots$, which comprise 72, 288, . . . $72n^2$, . . . amino acid residues. Figs. 2 and 3 show models of C_1 and C_2 in which the cyclol fabric is represented by the median hexagons. These models have 4 hexagonal

p.973

(3) It is found that the molecular weights of proteins are not distributed at random, but fall into a sequence of widely separated classes, the molecular weights in one class varying by as much as 15 per cent from a mean value. This is interpreted to mean

It is, however, suggested for consideration in the future, that the group of proteins with molecular weights ranging from 33,600 to 40,500[10] are closed cyclol molecules of type C_2. This molecular weight class is of particular importance, since Svedberg has suggested that very many, possibly most, other proteins have molecular weights which are multiples of (say) 36,000.

蛋白質の残基数は$72n^2$

72

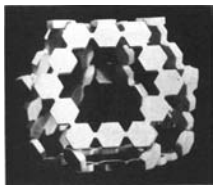


Fig. 2.

288

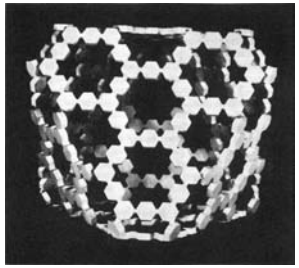


Fig. 3.

蛋白質の分子量は17600n

19

Stick model of the cyclol C1 protein structure

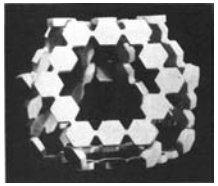
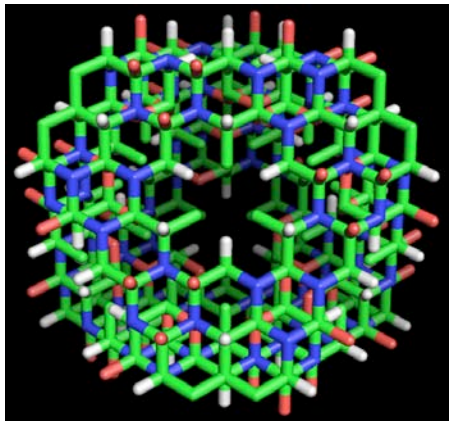


Fig. 2.



http://en.wikipedia.org/wiki/Cyclol

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(key paper)

Astbury...

WT Astbury, *Trans. Faraday Soc.* (1938) 34, 378-388

THE FOURTH
SPIERS MEMORIAL LECTURE.

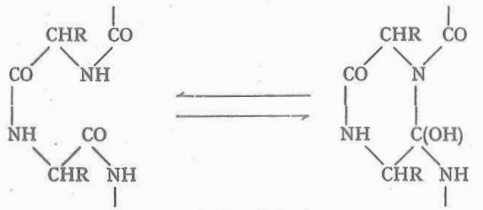
X-RAY ADVENTURES AMONG THE PROTEINS.

By William Thomas Astbury, M.A., Sc.D., F.Inst.P.

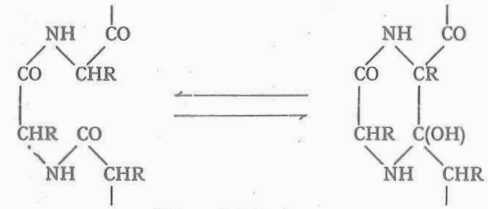
" Amino-acids in chains
Are the cause, so the X-ray explains,
Of the stretching of wool
And its strength when you pull,
And show why it shrinks when it rains."
A. L. Patterson.

p.385

The task of protein studies now is to try to find out the form or forms of protein folds. A possible solution for keratin and myosin that fits in well with the X-ray and elastic data is that they are hexagonal and closed by a lactam-lactim or a keto-enol interchange, thus : —



Lactam-lactim Interchange.



Keto-enol Interchange.

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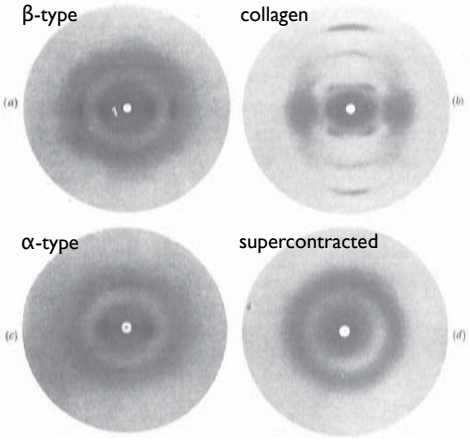

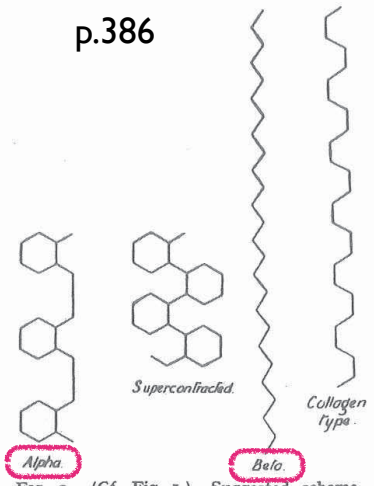

FIG. 2.—(*Cf.* Fig. 1.) Suggested scheme for the four principal states of fibrous proteins.

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「当時の」知識…

one-sixteenth arginine residues, and so on, and suggest that the smallest possible number of residues per molecule is $2^6 \times 3^3$, or 2×288 (molecular weight about 68,000), corresponding to hæmoglobin and fibrin. However that may be, one thing seems clear enough, that we are now on the verge of something very fundamental indeed in protein theory, and the moral value alone of such stoichiometrical discoveries as these is immense. Exact analyses of the proteins, though always laborious, need no longer be the thankless tasks they have been. Every possible reliable observation now is urgently needed and must sooner or later be fitted into the puzzle. Above all, *complete* analyses of single proteins are necessary. . . .

Paulingの論証

key paper

Pauling & Niemann, *JACS* (1939) 61, 1860-1867

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 708]

The Structure of Proteins

BY LINUS PAULING AND CARL NIEMANN

Paulingの道具

The theory of chemical binding

「論争」…

p.1860

structural elements of the cyclol type. Until recently no evidence worthy of consideration had been adduced in favor of the cyclol hypothesis.

examined the X-ray arguments and other arguments which have been advanced in support of the cyclol hypothesis, and have reached the conclusions that there exists no evidence whatever in support of this hypothesis and that instead strong evidence can be advanced in support of the contention that bonds of the cyclol type do not occur at all in any protein. A detailed discussion of

p.1861

theory. We wish to point out that the evidence adduced by Wrinch and Langmuir has very little value, because their comparison of the X-ray data and the cyclol structure involves so many arbitrary assumptions as to remove all significance from the agreement obtained. In order to at-

p.1861

mental diagrams would in itself make the argument advanced by Wrinch and Langmuir unconvincing; the fact that many other parameters were also assigned arbitrary values removes all significance from their argument.

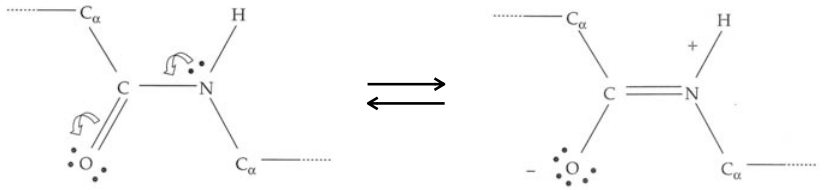
p.1866

There can be found in the papers by Wrinch many additional statements which might be construed as arguments in support of the cyclol structure. None of these seems to us to have enough significance to justify discussion

so involved, the arguments are so lacking in rigor, and the conclusions are so indefinite that it would not be possible to present the experimental evidence at the basis of our ideas of protein structure[48] in a brief discussion. In the following para-

共鳴構造

Resonance



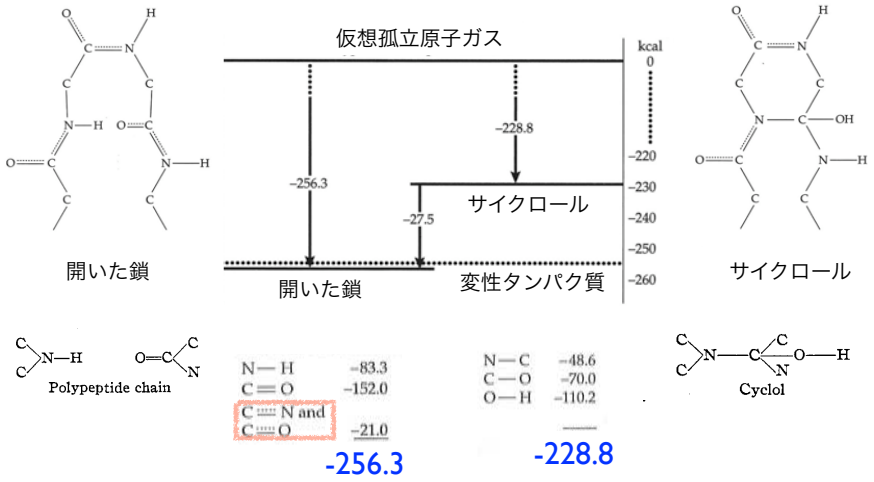
1.49Å 1.27Å

共鳴構造のポイント

ペプチド結合の安定化

21 kcal/mol 安定

化学結合エネルギーとサイクロール



p.1867

Crystal structure investigations have shown that in general the distribution of matter in a molecule is rather uniform. A protein layer in which the peptide backbones are essentially coplanar (as in the β-keratin structure) has a thickness of about 10 Å. If these layers were arranged as surfaces of a polyhedron, forming a cage molecule, there would occur great steric interactions of the side chains at the edges and corners. (This has been used above as one of the arguments against the C_2 cyclol structure.) We accordingly believe that *proteins do not have such cage structures*.[50] A compact structure for a globular protein might involve the superposition of several parallel layers, as suggested by Astbury, or the folding of the polypeptide chain in a more complex way.

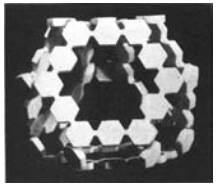


Fig. 2.

p.1867

Summary

It is concluded from a critical examination of the X-ray evidence and other arguments which have been proposed in support of the cyclol hypothesis of the structure of proteins that these arguments have little force. Bond energy values and heats of combustion of substances are shown to lead to the prediction that a protein with the cyclol structure would be less stable than with the polypeptide chain structure by a very large amount, about 28 kcal./mole of amino acid residues; and the conclusion is drawn that proteins do not have the cyclol structure. Other arguments leading to the same conclusion are also presented. A brief discussion is given summarizing the present state of the protein problem, with especial reference to polypeptide chain structures.

PASADENA, CALIF. RECEIVED APRIL 22, 1939

key paper

Wrinchの反論

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY]

The Geometrical Attack on Protein Structure

BY DOROTHY M. WRINCH

JACS (1941) 63, 330-333

「反論」…

p.330

It is unnecessary at the present time to state the case for the cyclol hypothesis, since authoritative accounts have already been given by Langmuir of the way in which the theory accounts satisfactorily for many of the well-known properties of the globular proteins.[1,2] In a recent summary,[3] however, Pauling and Niemann repeat a number of statements purporting to disprove the theory already made by other writers. Attention must therefore be directed to a number of publications in which these criticisms have already been discussed, at least so far as their scientific importance appeared to warrant.[2,4-9] We then proceed to

(a) In my first studies of possible protein structures, very exacting metrical conditions were adopted, mainly in order to demonstrate in a simple manner the possibility of handling problems of protein structure by strictly mathematical methods. These, it appears from crystallo-

p.331

of amino acid residues. It is my opinion that both claims (1) and (2) were unfounded, and that the suggested deduction therefore falls to the ground.

(2) Little or nothing is known as to the structure of any denatured protein, nothing as to the structure of the denatured trypsin under discussion. It is assumed by Pauling and Niemann that denaturation on the cyclol theory means the opening of all cyclol bonds. This is not the case.

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particular cases. Particularly I question the validity of (1) assuming that the C–CH_2 bonds have the same energy in both structures, (2) assuming the C–O bonds in cyclols have an energy as small as in the primary alcohols. These uncertainties appear to me to make the calculation valueless, except in so far as it calls attention

p.333

It must be concluded that no case has been made out for deducing that the cyclol theory is false on the basis of Anson and Mirsky's figure for the heat of denaturation of trypsin; further, that

p.333

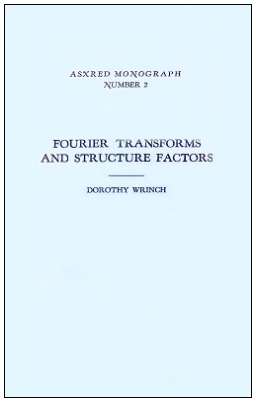
Summary

Arguments against the cyclol hypothesis, which have been collected by Pauling and Niemann in a recent article, are examined. It is found that they do not disprove it. In particular their statements purporting to prove that a protein with the cyclol structure would be less stable than the polypeptide chain structure by a very large amount is examined and found to be unproven.

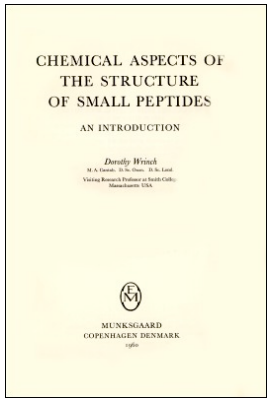
BALTIMORE, MD. RECEIVED APRIL 30, 1940

その後のWrinchと蛋白質結晶学

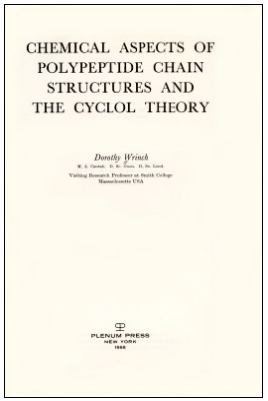
1946



1960



1965



http://www.agnesscott.edu/lriddle/women/wrinch.htm

key paper

ヘリカルモデルの提案

Polypeptide chain configurations in crystalline proteins

By Sir Lawrence Bragg, F.R.S., J. C. Kendrew and M. F. Perutz
Cavendish Laboratory, University of Cambridge

(*Received* 31 *March* 1950)

Proc. Roy. Soc. London (1950) A203, 321-357

α-helix の提案

「紙」と手による論考

THE STRUCTURE OF PROTEINS: TWO HYDROGEN-BONDED HELICAL CONFIGURATIONS OF THE POLYPEPTIDE CHAIN

By Linus Pauling, Robert B. Corey, and H. R. Branson*

Gates and Crellin Laboratories of Chemistry,
California Institute of Technology, Pasadena, California†

Communicated February 28, 1951

Proc. Natl. Acad. Sci. USA (1951) 37, 205-211

α-helix の実験的検証

No. 4261 June 30, 1951 NATURE 1053

NEW X-RAY EVIDENCE ON THE CONFIGURATION OF POLYPEPTIDE CHAINS

M. F. Perutz

Cavendish Laboratory,
University of Cambridge.

Nature (1951) 167, 1053-1054