Appendix 2

The Question of Preference for the Same Hand

In this appendix, we will give more of the details on which the conclusions in chapter 3 were based, regarding the matter of preferential linking of amino acids of the same hand.

Left- and Right-Handed Amino Acids Can Link

Are the two forms of the amino acids shaped so that any of them could unite, whether they are L- or D- in type, as far as contour is concerned? The answer is yes, according to California Institute of Technology's veteran researcher, James Bonner. Whether left- or right-handed, any amino acid can be linked with any other of either hand.[1]

Sidney W. Fox, at the University of Miami, said he was inclined to agree with Bonner on this.[2] The resulting shape of the chain, whether it spirals and how it folds, will be entirely different, but numerous polymers or chains have been put together containing both L- and D-amino acids of a variety of types, including some with the largest side chains, such as the amino acids tyrosine and phenylalanine.[3]

Among others, Dr. Fox believes that an all-one-handed chain is "thermodynamically more stable" than one composed of both forms.[4] The stability is increased by hydrogen bonds between the turns of the spiral.

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[1] James Bonner, telephone conversation, April, 1971.
Bonner is noted among other things for his discoveries in the biology of development, and the role of certain proteins (histones) in gene repression.

[2] Sidney W. Fox, telephone conversation, April, 1971.

[3] C. M. Venkatachalam and G. N. Ramachandran, "Conformation of Polypeptide Chains," *Annual Review of Biochemistry,* Vol. 38 (1969), pp. 627-690.

[4] Fox, personal communication, 1971.

The big problem, which has no natural solution in sight, is how to get such a chain even the first time. It is important to keep in mind that natural selection could not logically have been operative at that stage (as shown in chapter 5).

This question of whether there would be "steric hindrance," or difficulty of opposite hands fitting together due to shape, was put to Dr. Linus Pauling at Stanford University. He mentioned that there was that possibility,[5] but for details he referred me to a section in one of his books. There he had written, "We have no strong reason to believe that molecules resembling proteins could not be built up of equal numbers of right-handed and left-handed amino acid molecules."[6]

On the same matter, Dr. Arthur Elliott, of the Biophysics Department at King's College in London, said that he knew of none of the common amino acids that could not be connected to any other opposite isomeric form.[7] Dr. Harry Block, at the University of Liverpool's Chemistry Department, was of the same opinion—he knew of no exceptions.[8] In other words, it is possible for any of the twenty amino acids to connect with any other of the same or opposite hand.

Linkups Under Primitive Conditions

Would linkups under the assumed conditions of primitive synthesis include both isomers? The experiments by Dr. Sidney Fox seem to indicate that if amino acid chains had formed naturally under those assumed early conditions, they would have had both left- and right-handed amino acids in the chains, instead of all L-monomers as proteins now contain.[9]

Will Opposites Join With Equal Ease?

At the present stage of experimental knowledge in this field, there is nothing like complete certainty on whether in general there is equal preference for either hand, or, if not, what degree of selectivity exists. Study of reports on experiments to date seems to warrant this conclusion: There is either equal

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[5] Linus Pauling, telephone conversation, April, 1971.

[6] Linus Pauling, *College Chemistry,* 3rd ed., (San Francisco: W. H. Freeman & Co., 1964), p. 731.

[7] Arthur Elliott, telephone conversation, April, 1971.

[8] Harry Block, telephone conversation, April, 1971.

[9] Sidney W. Fox, in *The Origins of Prebiological Systems and of Their Molecular Matrices* (New York: Academic Press, 1965), pp. 361-382.
Also: personal communication, 1971.

probability *on the average,* or else some limited degree of preference usually unnoticed.

In the literature, there are hundreds of write-ups of experiments where D- to L- linkups were made and vice versa. Almost never does one find any mention made of any more difficulty in joining opposite hands than in linking similar isomers. There have been some rare exceptions to this general picture which we will look at in more detail and which seem to indicate a degree of selectivity in some particular joinings.

An article in *Biopolymers* by E. Klein et al. is a good example of a description of matter-of-fact linking of opposites.[10] In this instance, the amino acid chains formed were "poly-D,L-leucine, co-D,L-methionine." Dr. Klein is a research scientist for Gulf South Research Institute at New Orleans. When I asked if he and his colleagues knew of any preference of L- for L- or D- for D- in their experiments, he said that if there was any selectivity, they had not detected it in their work which often involves the joining of preformed blocks of each isomer within the same chain.[11]

One of the best ways to get an understanding of this matter is to work with models of the amino acid residues. If ready-made atomic models are not available, it is possible to get by with styrofoam balls or other round objects. It is very important to make the models to scale. Attention must be given to comparative sizes of the different atoms, length of bonds, bond angles, and bonds which allow rotation.

A convenient source for most information one would need is Pauling's *The Chemical Bond,* perhaps available in your local library. Dr. Pauling gives tables of effective radii or size of the atoms (van der Waals radii), bond lengths, angles, and positions.[12]

In general, some rotation is allowed on the single bonds. In the backbone of an amino acid chain, these bonds which allow rotation are on either side of the alpha carbon atom.[13] There is also rotation permitted on single bonds in the side chains, especially in the case of "methyl" groups.

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[10] E. Klein et al., "Permeability of Synthetic Polypeptide Membranes," *Biopolymers,* Vol. 10, No. 4 (April, 1971), pp. 647-655.

[11] E. Klein, telephone conversation, June, 1971.

[12] Linus Pauling, *The Chemical Bond* (Ithaca, N.Y.: Cornell University Press, 1967), pp. 136, 152, 229.

[13] Douglas Poland and A. Scherage, "Theory of Noncovalent Structure in Polyamino Acids," *Poly-Alpha-Amino Acids,* ed. Gerald Fasman (New York: Marcel-Dekker, Inc., 1967), p. 396.

Where two amino acids are joined, the four atoms involved (C'O-NH) are "co-planar" or in the same plane, and therefore are more or less fixed or rigid. For this reason, we have found it logical to make the models in two separate units. The peptide linkage group can be one item (the co-planar C'O-NH just mentioned). The other consists of the alpha carbon, with its hydrogen atom and R group.

You can get a reasonably complete understanding by selecting a few representative shapes of the twenty types and experimenting with those. Tryptophan, phenylalanine, and proline are good examples of the more complex ones. P. K. Ponnuswamy and V. Sasisekharan, of the University of Madras in India, give helpful information on positions of the atoms.[14] It is out of the question to try all conformations, for the number is virtually infinite. We might mention that electrostatic forces may in some cases restrict bond rotation to a degree, but this does not seem to be a barrier to more or less equal ease of fit.

If one experiments with molecular models, the impression grows that the probability is *approximately equal, on the average,* that opposites will fit as well as those of the same hand.

Some Indications of Preferential Linking

A few experimenters have reported cases where one isomer would join up easier than its opposite, as a chain is polymerizing.

Dr. Akiyoshi Wada, at the Department of Physics of Tokyo University, discussed the implications of certain experiments in the 1950's by Doty, Lundberg, Blout, and others.[15] These experiments seemed to show that a preformed chain consisting of L-residues begins reaction more rapidly with another L-unit than with the opposite isomer, in the case of polymers of gamma-benzyl-glutamate NCA, an amino acid altered for experimental purposes.[16]

Important work on the same question was done by C. H. Bamford and Harry Block, who are at the University of Liver-

[14] "Studies on Conformation of Amino Acids," *Biopolymers,* Vol. 10, No. 3 (March, 1971), pp. 565-582.

[15] R. D. Lundberg and Paul Doty, "A Study of the Kinetics of the Primary Amine-initiated Polymerization of N-Carboxy-anhydrides with Special Reference to Configurational and Stereochemical Effects," *American Chemical Society Journal,* Vol. 79 (1957), pp. 3961-3972. Also:
E. R. Blout and M. Idelson, in *American Chemical Society Journal,* Vol. 78 (1956), pp. 3857, 3858.

[16] Akiyoshi Wada, "Chain Regularity and Dielectric Properties of Poly Alpha Amino Acids in Solution," *Polyamino Acids, Polypeptides, and Proteins,* ed. M. A. Stahmann (Madison, Wis.: University of Wisconsin Press, 1962), pp. 131-146.

pool.[17] Put in simplest terms, they found indications that an L-amino acid was five or six times more likely than a D-unit to connect at the end of a preformed L-chain, and vice versa, in certain circumstances.

The synthetic polymers used in much of this type of work are not made of simply the amino acid molecules. Instead, an attachment consisting of a carbon ring and several other atoms has been added to the usual side chain of glutamic acid, which is one of the twenty amino acids. It seems likely that this long and bulky extra portion of the side chain might cause more steric hindrance than a normal amino acid would have. (Glutamic acid and aspartic acid are amino acids which have an acidic group in their side chains, and this must be protected from reaction with other molecules in the solution. The protection of these and other reactive groups—including the ends of amino acids—is one of the main problems in peptide chemistry in the laboratory.)[18]

Dr. Elliott called my attention to other polymerizations that are done, e.g., poly-alanine, where extra attachments are not left on the amino acid residues in the chain.[19] I later asked Dr. Block if, in cases of the kind just mentioned, any selective bonding of L- to L- as compared to D- to L- had been observed. He indicated that experiments with the amino acids alanine and phenylalanine did seem to show a preference. He did not have data on the degree of preference, but said there must have been some selectivity, because there were, in the resulting chains, blocks of L- and blocks of D-residues.[20]

Applying probability theory to this matter of "blocks" would seem to lead to this result: as soon as—by chance—one isomer happened to get four (apparently that is the critical number) in a row, probability would favor that type, by a certain factor. On average, the opposite would eventually show up, and then there would be equal probability until one isomer or the other got four in series again. The final result would not favor either hand as to total residues, but each would have the same average number and length of blocks and single residues.

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[17] C. H. Bamford and H. Block, "The Polymerization of Alpha Amino Acid N-Carbonic Anhydrides," *Polyamino Acid , Polypeptides, and Proteins,* ed. M. A. Stahmann, (Madison, Wis.: University of Wisconsin Press, 1962), pp. 65-78.

[18] T. Wieland and H. Determann, "The Chemistry of Peptides and Proteins," *Annual Review of Biochemistry,* vol. 35, pt. II (1966), pp. 656-658.

[19] Telephone conversation, 1971.

[20] Telephone conversation, 1971.

On the question of whether the length of the helix increased the preference, Professor Block said it did not. (Drs. Elliott and Block have authored reports on such subjects.[21])

A mysterious reaction was reported by Bamford and Block,[22] in which the addition of lithium perchlorate to a reaction mixture eliminated optical specificity, leaving both forms reacting at the same rate with a 15-mer[23] helix as would be expected with a short 3-mer chain.

This was part of experiments mentioned earlier which involved gamma-benzyl-glutamate N-carboxy-anhydride. In those experiments, the same handed amino acid was thought to be five or six times as fast as the opposite hand in joining the end of a preformed helix. The experimenters reasoned that the lithium perchlorate reduced hydrogen bonding of the "NCA" to the helix. The result seemed to show "that the specificity may, in part, be connected with adsorption." (Adsorption is the attaching of one atomic or molecular entity to another through electrostatic forces.)

As a result of certain of the experiments by Doty, Bamford, Block, and others, some came to believe that this stereoselectivity was a result of steric hindrance or other conflict with the preformed helix. Dr. Fred D. Williams, at Michigan Technological University, doubts, however, that this is the cause. He and his co-workers report polymerization of the same glutamic acid complex in experiments where results seemed to indicate a selectivity even before a helix was formed.[24]

This is another case where one may wonder if the bulky artificial side chain mentioned earlier may be affecting the outcome.

Dr. Williams told me of quite opposite results in their recent experiments with D,L-alanyl-isoleucine.[25] These two amino acids exhibited a *crosswise* selectivity (D- to L- instead of L- to L- preference)!

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[21] Arthur Elliott, "X-ray Diffraction by Synthetic Polypeptides," *Poly-Alpha-Amino Acids,* ed. Gerald D. Fasman (New York: Marcel-Dekker, 1967), pp. 1-64. Also:
Bamford and Block, "Polymerization."

[22] Bamford and Block, "Polymerization."

[23] *Mer* meaning part or unit—in this case, amino acid residue. Thus, *monomer* means one unit, *polymer* means many units.

[24] Fred D. Williams, M. Eshaque, and Ronald D. Brown, "Stereoselective Polymerization of Gamma Benzyl Glutamate NCA," *Biopolymers,* Vol. 10, No. 4 (April, 1971), pp. 753-756.

[25] Telephone conversation, June, 1971.

Shröder and Lübke in West Berlin reported a similar reverse preference in "cyclo" polymers of glycine and D,L-leucine.[26]

As can be seen, the picture is far from final on the preference factor, at this writing. There are many variables which enter into the reaction potentials. The type of solvent used, temperature, pH reading, protecting attachments, all these can affect the joining. The activating intermediates which must be used to bring about the linking also may complicate the picture.

Some chemical reactions, moreover, bring about "racemization" as a side-effect—changing some amino acids themselves to the opposite isomers.[27]

Then there is the problem of trying to "read" the results. When we recall that biochemists are working with molecules far too small for the ordinary laboratory microscope, it indicates how hard it can be to tell exactly what happened in a reaction, and to what degree. The results must be discovered by roundabout means. Sometimes a bit of guesswork has to enter in. Later experiments may disprove the tentative conclusions of the experimenter.

Equal Probability of Opposites Joining on the Average

A fairly good case might be made for the idea that there is equal probability of opposite antipodes linking, as an average of all types. These are some of the indications which point in that direction:

1. Numerous reports of D,L-chains with no mention of any stereo-selective factor, and specific statements by experimenters such as Dr. E. Klein that they have not noted any such preference.

2. The reports of a reverse preference (L- for D-) in some experiments, which may tend to balance reports of L- for L-preference in others.

3. The evidence from experimenting with models of amino acid residues, which seems to favor equal ease of fit on the average.

4. Opinions of prominent researchers such as Bonner and Fox who, in conversation with the author, made no mention of selectivity when commenting on the ability of all to fit.

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[26] Eberhard Shröder and Klaus Lübke, *The Peptides* (New York: Academic Press, 1965), pp. 274, 275, 319-326.

[27] Abraham White, Philip Handler, and Emil L. Smith, *Principles of Biochemistry*, 3rd ed. (New York: McGraw-Hill, 1964), p. 92.

5. It seems likely that in a presumed primitive environment prior to the existence of life, the numerous variables that affect reactions would by chance be as likely to favor one type of hookup as another. Apparently, no steric reasons would prevent average equal ease of fit. There is no reason to suppose that nature would by chance provide the same highly specific reactions and conditions that a biochemist might choose from his artificially prepared supply.

Since there have been more reports of L- for L- and D- for D- preference than of opposites, however, we will consider that type of preference as one of the possible conclusions on the way things are on the average.

We may conclude, then, that any amino acid can link with any other, as to fit, and that opposites are either (1) equally likely to unite, or (2) in some cases there may be a preference for the same enantiomer, up to 6/7 probability. In chapter 4 the laws of chance are applied to both of these possibilities.

In both cases, the outcome is conclusive. Chance cannot at all account for even the comparatively simple fact that only left-handed amino acids are used as the components of naturally occurring regular proteins.