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Stereospecificity of the Genetic Code

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Summary. A sterical correlation of the amino acids to their anticodon nucleotides is given. The main principle is the intercalation of the amino acid and the binding of the aliphatic amino acid hydrogen atoms through hydrogen bonds to the π-electrons of the bases. The amino groups of the bases and the ribose phosphate chain are additional binding sites for the amino acid. The strength of these hydrogen-π bonds is considerably increased by the protonation of the carboxyl group of the amino acid. Such a protonation occurs in esterification processes and gives in these reactions the possibility of an activated complex where the proposed complementarity is also energetically favoured. Evolutionary considerations show an uncomplicated way from the chemical reactions of prebiologically formed organic molecules to a living system.

Key words: Genetic Code — Stereospecificity — NMR-Measurements — Evolution.

Introduction

The genetic code as the basis of protein biosynthesis implies the problem of stereospecificity. The biological correlation of an amino acid with a trinucleotide is not possible without stereospecific interactions either on the monomer or on the macromolecular level.

According to the universal concept of "complementarity of biological structures" (Pauling and Delbrück, 1950) the nucleic acids should be templates for the amino acids, as proposed by Gamow (1954) (Woese, Dugre, Dugre, Kondo and Saxinger, 1966a).

Crick opposed: "... If one considers the physico-chemical nature of the amino acid side chains we do not find complementary features on the nucleic acid. Where are the knobby hydrophobic surfaces to distinguish valine from leucine or isoleucine? Where are the charged groups in specific positions, to go with the acidic and basic amino acids? ..." (Crick; as quoted in Hoagland, 1960). Or according to Watson's formulation: "There is no specific affinity between the side groups of many amino acids and the purine and pyrimidine bases found in RNA. For example, the hydrocarbon side groups of the amino acids alanine, valine, leucine, and isoleucine do not form hydrogen bonds and would be actively repelled by the amino and keto groups of the various nucleotide bases ..." (Watson, 1965).

With increasing evidence for the adaptor hypothesis which Crick proposed as result of his postulate of the impossibility of amino acid-nucleic acid regognition, this postulate became a more or less unquestioned dogma (Woese *et al.*, 1966a). But this hypothesis shifts the problem of biological stereospecificity only from the monomer level of interaction between amino acids and nucleotides to the macromolecular level of interactions between high molecular proteins and nucleic acids, leaving the phenomenon of molecular stereospecificity unexplained. It can also be shown that the existence of adaptor molecules is not necessarily connected with the absence of amino acid-nucleic acid interactions.

Therefore one should reexamine the statements of Crick and Watson by formulating a new question: Is it true that the hydrocarbon side groups of the amino acids cannot form hydrogen bonds and do nucleic acids possess no knobby hydrophobic surfaces to which such hydrogen bonds can be formed?

The demand for hydrogen bonds as basis of molecular biological stereospecificity is due to their defined geometry and preferred orientation (Kollman, 1972). It makes them suitable to cause exact sterical arrangements between two molecules. Regardless of these general valid properties, hydrogen bonds can accept different strength, reaching from the relatively strong hydrogen bonds building water structures and nucleic acid base pairing to the weak hydrophobic interactions of positivated aliphatic hydrogen atoms to electron donors (Pauling, 1960).

Such weak hydrogen bonds, especially those of haloforms, have been examined with the help of different techniques: Nuclear magnetic resonance (Lichter and Roberts, 1970; Lin and Tsay, 1970; Korinek and Schneider, 1957; Wiley and Miller, 1972), infrared spectroscopy (Lascombe, Devanie, and Josien, 1964; Whetsel and Kagarise, 1962), freezing point diagrams (Korinek and Schneider, 1957), calorimetry (Findlay, Keniry, Kidman and Pickles, 1967; Tamres, 1952), and X-ray crystallography (Dahl and Hassel, 1970; Hassel, 1970). These results indicate stoichiometric complexes due to hydrogen bonding of aliphatic hydrogen atoms with lone-pair electrons and aromatic systems. The hydrogen bond to an aromatic system, e.g. of chloroform to benzene, is formed in such a manner that the dipole axis of chloroform is along the sixfold axis of symmetry of the benzene ring with the proton nearest the benzene (Lin and Tsay, 1970). The strength of such bonds of aliphatic hydrogen atoms to n-donors lies between 1 and 4 kcal/mole (Wiley and Miller, 1972) and to π-donors in the order of 1 kcal/mole (Ronanyne and Williams, 1969).

Generally the interaction energy depends on the positive charge of the hydrogen atom and on the lone-pair or π-electron charge distribution of the atom or molecule to which this bond is formed (Kollman and Allen, 1972). Aliphatic hydrogen atoms of amino acids should also be able to form

such hydrogen bonds. In this case the amino- and carboxyl groups, especially their degree of protonation, might be of great importance for the strength of these bonds.

The sterical principle of how two macromolecules can achieve stereospecific binding could lie in the fitting and binding of the surfaces of the molecules to one another (Adler, Beyreuther, Fanning, Geisler, Gronenborn, Klemm, Müller-Hill, Pfahl and Schmitz, 1972). But since these surfaces contain mainly hydrophilic groups which bind well to water, the hydrophilic bonds between the molecule surfaces will again be easily dissolved by water. Thus additional hydrophobic bonds achieved through partial intercalation of the molecules may contribute to complex stability. In the case of intercalation of aliphatic and aromatic compounds into a nucleic acid, the π-electron systems of the bases become the knobby hydrophobic surfaces as requested by Crick.

It will be the purpose of this paper to examine whether the concept of intercalation under the aspect of hydrogen bonding of the aliphatic amino acid hydrogen atoms to the π-electrons of the bases is able to contribute to the stereospecificity of the genetic code. Therefore this paper falls into three parts: Firstly it will be examined if a sterically reasonable correlation is possible, secondly if the considered hydrogen-π bonds can be strong enough, and finally, what evolutionary aspects—in regard to the evolution of the translation mechanism and the adaptor hypothesis—arise.

1. The Steric Relationship between Amino Acids and Trinucleotides

The genetic code gives an unequivocal correlation between amino acids and trinucleotides (Table 1).

A steric relationship as basis of the genetic code can be obtained when the amino acid is intercalated between the first two anticodon bases (Melcher, 1970). In this arrangement the aliphatic hydrogen atoms of the amino acid form hydrogen bonds to the π-electrons of the bases. This hydrogen bonding is essentially electrostatic in character (Lin and Tsay, 1970), so that the binding strength will increase with increasing positive charge of the hydrogen atom and with increasing π-electron density of the bases. Therefore the nucleotides will show, according to the series $U < C < G < A$, increasing tendency to bind to aliphatic hydrogen atoms and thus to interact with amino acids. This order of the bases can be derived from decreasing length of bond distances of the aromatic rings, which is a measure for the degree of π-bonding (Donohue, 1968), from intermolecular shielding values derived through MO calculations (Giessner-Prettre and Pullman, 1970), and electron affinities (Bodor, Dewar and Harget, 1970) (Table 2) (Wagner, 1972). Theoretical calculations sometimes show a change

Table 1. The genetic code

Codon-nucleotide 1st	2nd	U	C	A	G
	3rd				
U	U	Phe	Ser	Tyr	Cys
	C	Phe	Ser	Tyr	Cys
	A	Leu	Ser	Term	Term
	G	Leu	Ser	Term	Trp
C	U	Leu	Pro	His	Arg
	C	Leu	Pro	His	Arg
	A	Leu	Pro	Gln	Arg
	G	Leu	Pro	Gln	Arg
A	U	Ile	Thr	Asn	Ser
	C	Ile	Thr	Asn	Ser
	A	Ile	Thr	Lys	Arg
	G	Met	Thr	Lys	Arg
G	U	Val	Ala	Asp	Gly
	C	Val	Ala	Asp	Gly
	A	Val	Ala	Glu	Gly
	G	Val	Ala	Glu	Gly

Ala = Alanine, Asp = Aspartic acid, Asn = Asparagine, Arg = Arginine, Cys = Cysteine, Glu = Glutamic acid, Gln = Glutamine, Gly = Glycine, His = Histidine, Ile = Isoleucine, Leu = Leucine, Lys = Lysine, Met = Methionine, Phe = Phenylalanine, Pro = Proline, Ser = Serine, Term = Termination codon, Thr = Threonine, Tyr = Tyrosine, Trp = Tryptophan, Val = Valine.

Table 2. Values of aromatic bases in connection with their π-electron density

Base	A	G	C	U
Sum of bond lengths of the pyrimidine ring[a]	8.13	8.25	8.22	8.27
Sum of bond lengths of the imidazole ring[a]	6.82	6.88		
Shielding values[b]	1.28	0.73	0.32	0.13
Electron affinities (eV)[c]	0.12	0.16	1.17	1.33

[a] Donohue (1968).
[b] Giessner-Prettre and Pullman (1970).
[c] Bodor, Dewar and Harget (1970).

in the order of guanine and adenine (Woese, Dugre, Saxinger and Dugre, 1966b).

In addition to these hydrophobic bonds the amino group of the amino acid can form hydrogen bonds to the lone-pair electrons of the amino groups of the bases. The lone-pair electrons of the keto groups are not

available for these bonds since they lie in the plane of the aromatic rings of the bases. Bonds of the amino acid to the ribose phosphate chain complete the possible interactions of an intercalated amino acid. According to these additional bonds the amino acids will be divided into four groups. The sterical considerations are based on model building with Kendrew atomic models (Cambridge Repetition Engineers). The obtained structures were photographed and redrawn in the following pictures.

1.1. Glycine, Proline, Alanine, Threonine, Serine, Cysteine

The amino acids of this group are determined through bonds between the amino groups, bonds between the aliphatic amino acid hydrogen atoms and the π-electrons of the bases, and hydrogen bonds of their hydroxy and mercapto groups to the 2′-hydroxy group of the second anticodon nucleotide.

The amino acids glycine and proline allow a clear decision in the relation of an amino acid to its anticodon, since proline will not fit into its codon cytosine-cytosine and glycine cannot fill out guanine-guanine. Both anticodon bases are the negative copies of their amino acids (Figs. 1 and 2).

Alanine also allows a sterical fitting to C-G (Fig. 3). Somewhat different is the case in the determination of threonine by U-G (Fig. 4). The difference between cytosine and uracil lies, according to Table 2, in the low tendency of uracil to form bonds with aliphatic hydrogen atoms, so that no specific sterically directed binding but only an unspecific shielding of the hydrophobic amino acid groups (of β- and γ-hydrogen atoms) against the surround-

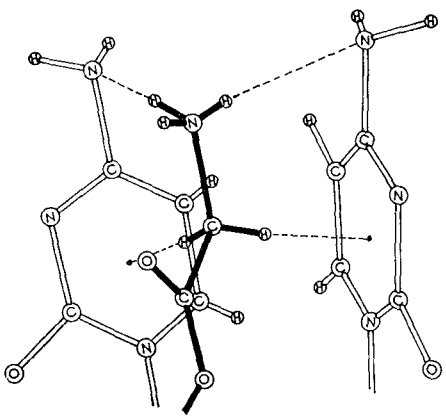

Fig. 1. Glycine, Anticodon: C–C–Pu, Py

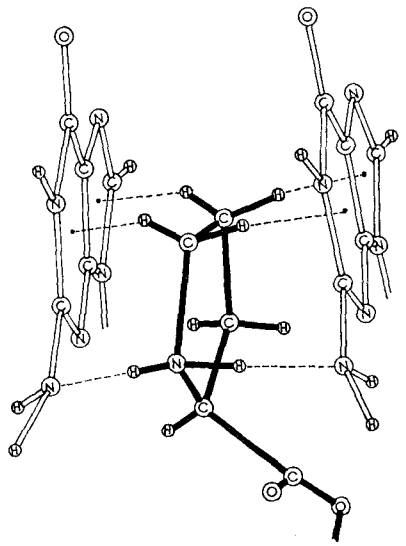

Fig. 2. Proline, Anticodon: G–G–Pu, Py

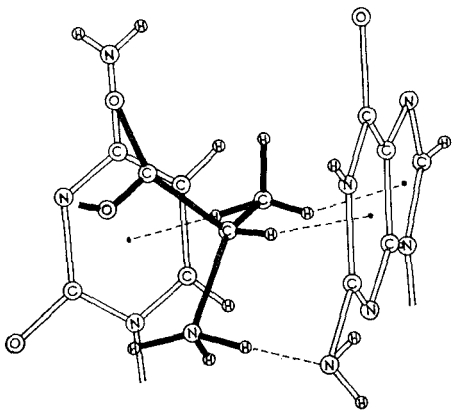

Fig. 3. Alanine, Anticodon: C–G–Pu, Py

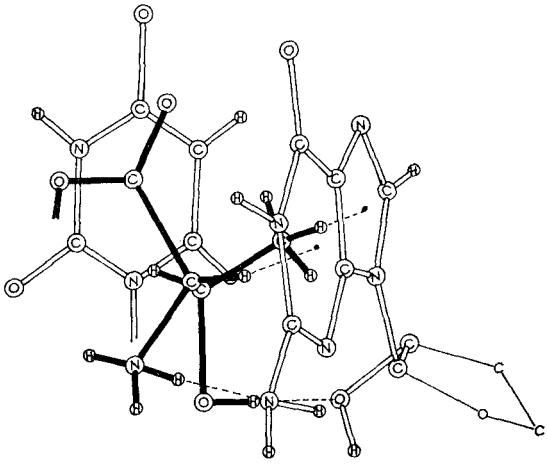

Fig. 4. Threonine, Anticodon: U–G–Pu, Py

ing water may result. This property of uracil can be also applied to explain the determination of other amino acids. The determination of threonine is thus nearly completely given through the second anticodon nucleotide with a bond of its hydroxy group to the 2′-hydroxy group of the guanosine residue (Fig. 4).

The situation is somewhat changed in the case of serine and cysteine. These amino acids are mainly bound to the first anticodon base adenine which fixes the skeleton of the amino acid. The second anticodon nucleotide determines the hydroxy- and mercapto-group. The steric difference between these two groups is the larger size of the mercapto group. Therefore the distance between the 2′-hydroxy group of the second nucleotide and the β-hydrogen atom of the amino acid which penetrates into the π-electrons of the second nucleotide has to be longer for cysteine. In regard to the smaller size of an imidazole ring compared to a pyrimidine ring this demand is fulfilled. If one assumes that the amino groups of the second anticodon bases are also bound to the amino groups of the amino acids which can be achieved through a rotation along the penetrating β-amino acid hydrogen atom, an additional enlargement of this distance for cysteine and a diminution for serine results. Such a binding of the amino groups is also possible for alanine (Fig. 3).

The sterical role of the third nucleotide will be explained on other amino acids. But it must be considered that it is not possible to disconnect entirely the genetic code from the evolutionary development and to treat it only from the sterical point of view. In the case of cysteine, which has only a purine residue as third anticodon nucleotide, this fact might have resulted from limiting influences through the evolution of the genetic code. The

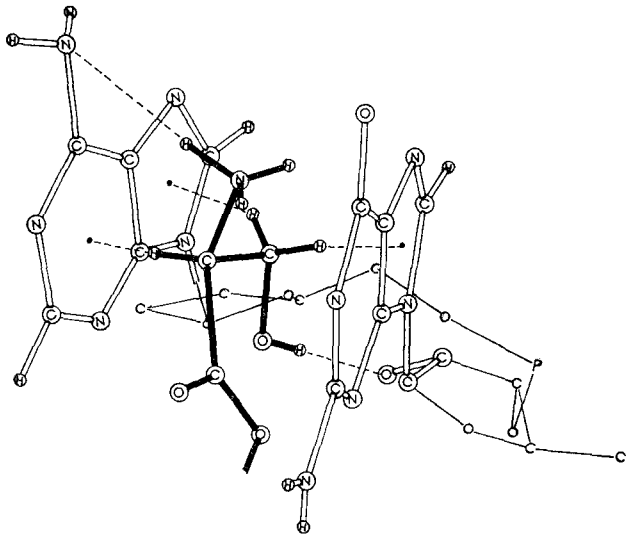

Fig. 5. Serine, Anticodon: A–G–Pu, Py

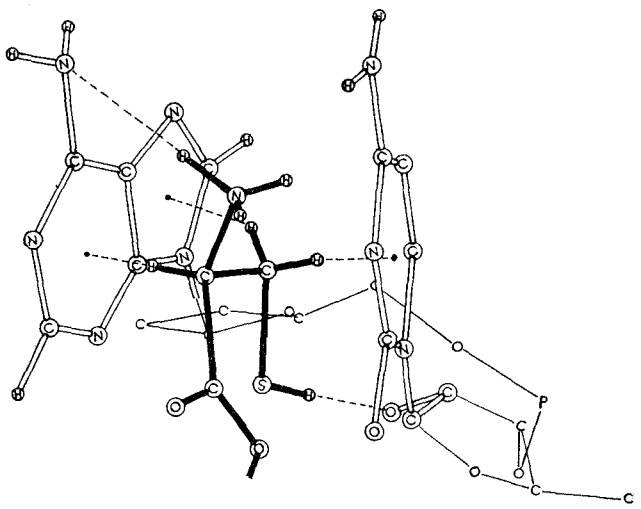

Fig. 6. Cysteine, Anticodon: A–C–Pu

same might be true in regard to the fact that serine has additional codons (A-G-Py). These anticodons contain only weak π-electron donors so that one has not necessarily to expect a steric relationship. Maybe for these nucleotides there was once another amino acid which has been excluded during an evolutionary phase and replaced by this amino acid (and arginine which codes for A-G-Pu).

1.2. Phenylalanine, Tyrosine, Tryptophan, Histidine, Methionine, Arginine

The aromatic amino acids possess π-electron systems which can bind to aliphatic hydrogen atoms, especially to the 2′-hydrogen atom of the second anticodon nucleotide which is positivated by the 2′-hydroxy group. Thus a sterical fixation of the amino acid in addition to the bonds of group 1.1 results. Another peculiarity of the aromatic amino acids is that always three nucleotides are determinant. The third anticodon nucleotide is able to lie on the two first anticodon nucleotides and to be bound by hydrogen bonds to the two bases so that a cagelike structure results into which the amino acid can be intercalated. The third nucleotide gives additional binding possibilities for aliphatic amino acid hydrogen atoms, so that only the ringsystem, purine or pyrimidine, is decisive for the distinction of the amino acids. In the case of the determination of tyrosine it is possible that a hydrogen bond between the phenolic hydroxyl group and the 2′-hydroxy group of the third anticodon nucleotide is formed (Fig. 7). It does not seem to be mere accident that most *t*-RNA^{phe}'s have this 2′-hydroxy group methylated (Zachau, 1969). This explains the determination of phenylalanine in distinction to tyrosine (Fig. 8).

In the case of tryptophan (Fig. 9) and histidine (Fig. 10) ionic bonds between the ring nitrogen atoms of the amino acid and the phosphate groups are possible.

The bases which determine methionine (UAC) also form a cage in which the amino acid can be arranged (Fig. 11). In the case of arginine, where only

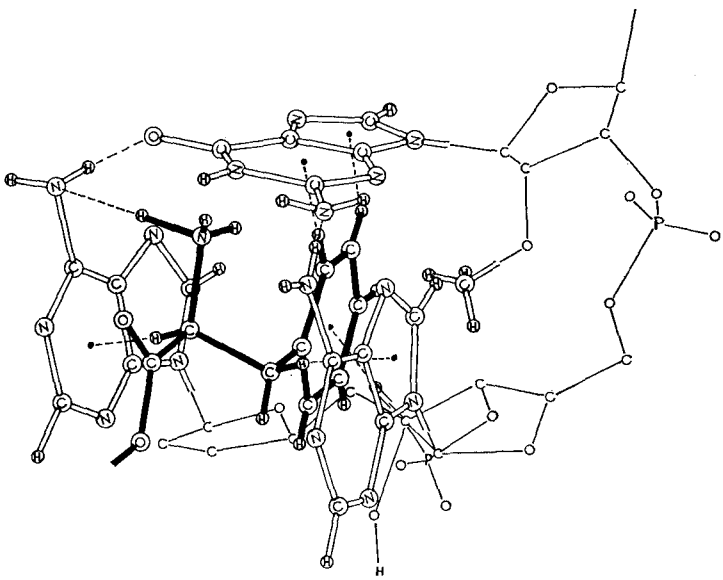

Fig. 7. Phenylalanine, Anticodon: A–A–Pu

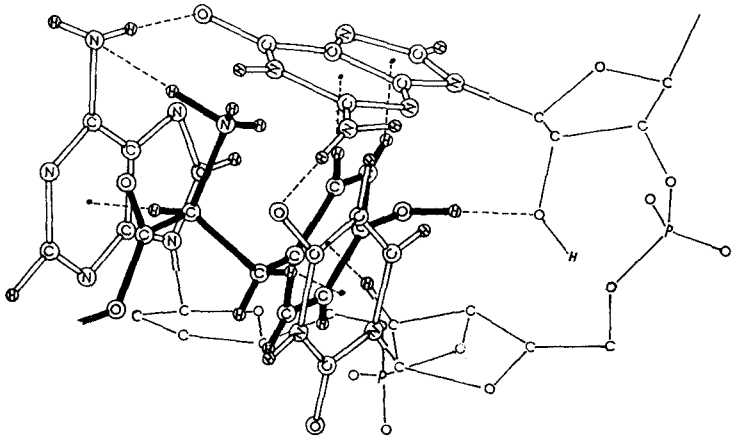

Fig. 8. Tyrosine, Anticodon: A–U–Pu

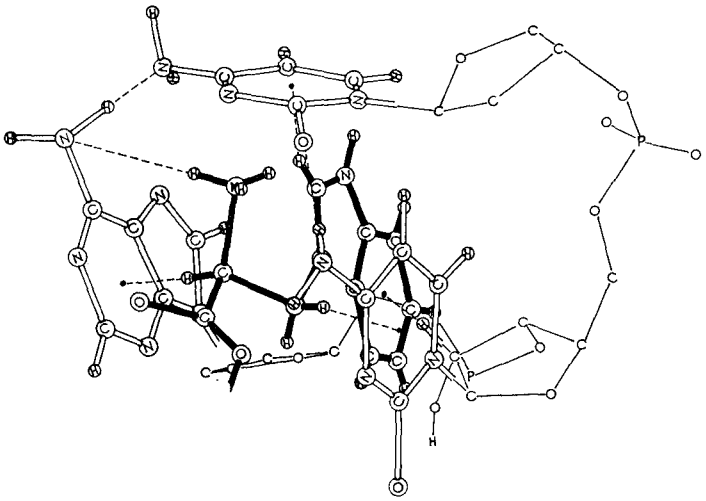

Fig. 9. Tryptophan, Anticodon: A–C–Py

G-C is necessary, an ionic bond to the phosphate group and two hydrogen-π-electron bonds are determinant beside the binding of the amino groups. This explains the preference for polyarginine to build complexes with guanosine-cytidine-rich nucleic acids (Yarus, 1969).

1.3. Valine, Leucine, Isoleucine

The amino acids with aliphatic branched side chains are only determined through hydrogen-π-electron bonds. The bases shield the side chains from

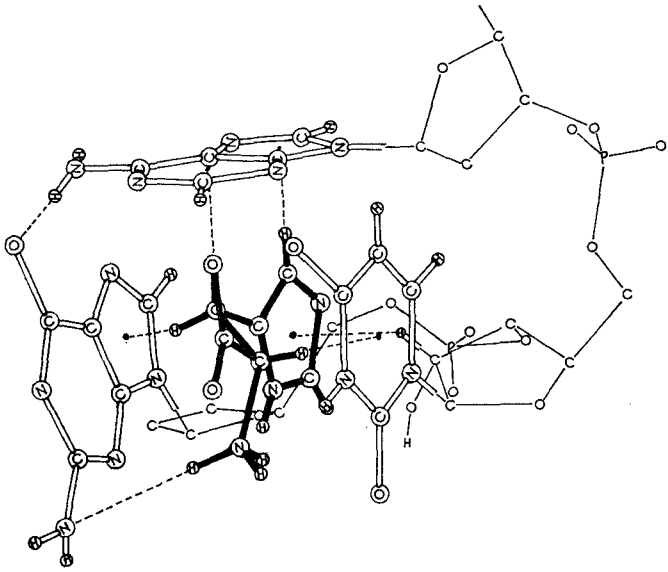

Fig. 10. Histidine, Anticodon: G–U–Pu

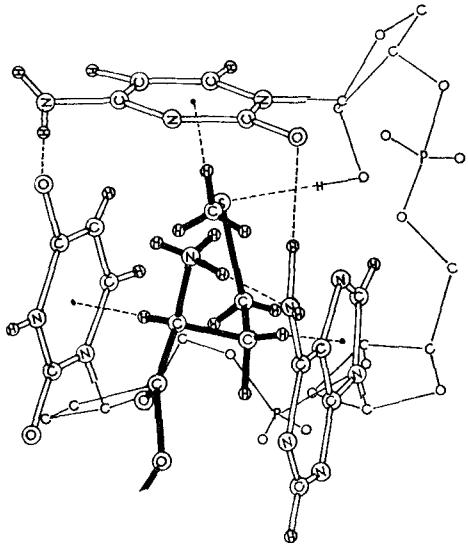

Fig. 11. Methionine, Anticodon: U–A–C

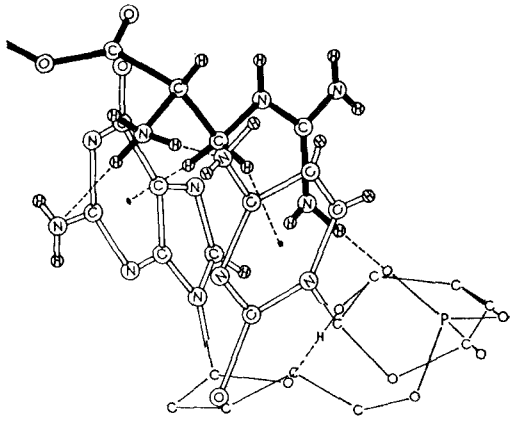

Fig. 12. Arginine, Anticodon: G–C–Pu, Py

water so that only the methyl groups reach into the water. The differences in the first anticodon base between valine (Fig. 13) and isoleucine (Fig. 14) can again be explained by assuming that uridine does not form a hydrogen-π-electron bond like cytidine, thus shielding a greater number of methylene groups from water. The intercalated leucine is shown in Fig. 15.

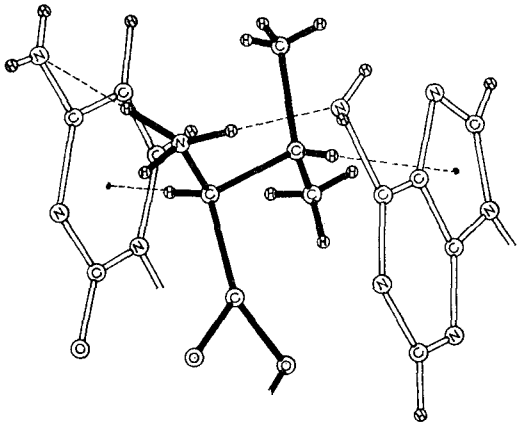

Fig. 13. Valine, Anticodon: C–A–Pu, Py

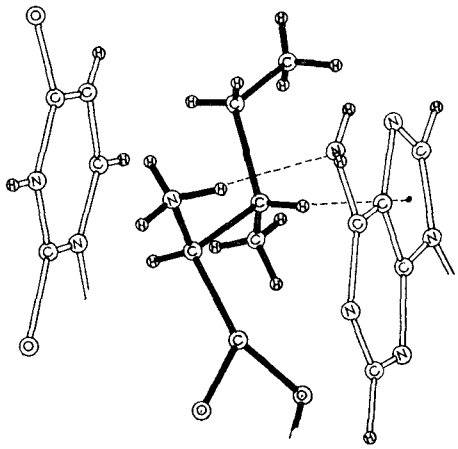

Fig. 14. Isoleucine, Anticodon: U–A–Pu, U

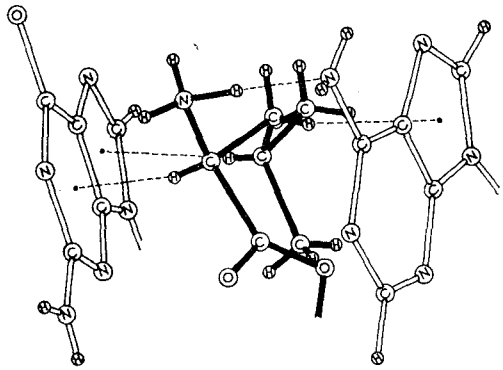

Fig. 15. Leucine, Anticodon: G–A–Pu, Py

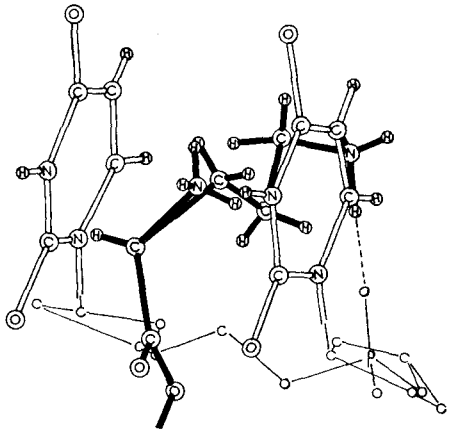

Fig. 16. Lysine, Anticodon: U–U–Py

1.4. Lysine, Aspartic Acid, Asparagine, Glutamic Acid, Glutamine

This group is characterized by the existence of an uridine as second anticodon nucleotide and in general by a second weak π-donor (uracil or cytosine) as first anticodon base. The amino acids have straight aliphatic side chains with relatively inert, not positivated, hydrogen atoms so that the interaction energy will be low and an intercalation becomes not very probable. It seems as if these amino acids fall through the above proposed determination scheme. So lysine is only determined by the binding of its terminal amino group to a phosphate group (Fig. 16).

The monoaminodicarboxylic acids and their amides may not be determined through intercalation according to their hydrophilic nature. It is

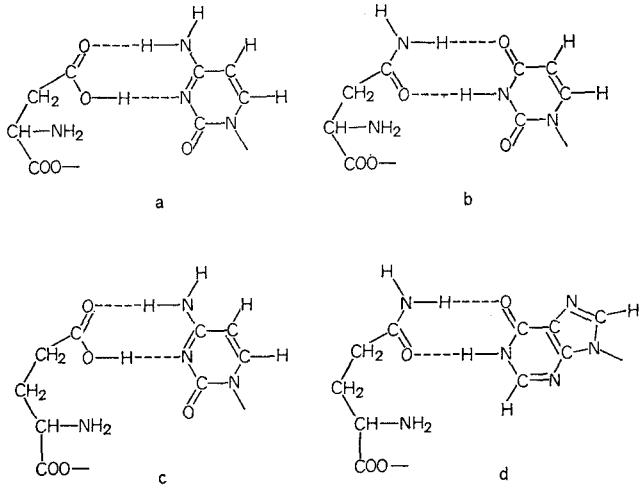

Fig. 17. a Aspartic acid, Anticodon: C–U–Pu. b Asparagine, Anticodon: U–U–Pu. c Glutamic acid, Anticodon: C–U–Py. d Glutamine, Anticodon: G–U–Py

possible that hydrogen bonding to the first anticodon base is the source of specificity (Fig. 17).

The anticodons AUU, ACU and AUC, which may also have coded originally for amino acids, may have taken over in the course of evolution other functions in the process of chain termination in protein biosynthesis (Model, Webster, and Zinder, 1969).

In accordance with the series $U < C < G < A$ of increasing π-electron density and tendency to bind amino acids by hydrophobic bonds, it is possible to divide the amino acids roughly into four groups according to the central codon-nucleotide (A-, G-, C-, and U-group) (Dickerson, 1971). Besides the role of the second nucleotide, one can find the domination of adenine as anticodon-nucleotide. Adenine as first anticodon-base joins the similar amino acids phenyl-alanine to tyrosine and serine to cysteine. Adenosine as second nucleotide (U-group) determines leucine, isoleucine and valine. These are amino acids with hydrophobic side chains which occur generally in the inside of globular proteins. Amino acids of the A-group (with an uracil as second anticodon base) have hydrophilic side chains (aspartic acid, asparagine, glutamine, glutamic acid, lysine). Amino acids of the C- and G-group are between those of the A- and U-groups; the C-group is perhaps more hydrophobic than the G-group (Orgel, 1972). Facts which on the other hand show the importance of hydrophobic interactions in the correlation of amino acids and trinucleotides in the genetic code.

An exact criterion is necessary to control if a given sterical correlation is a real one, since models can be easily manipulated (Woese *et al.*, 1966a).

Since the here presented correlation of di- and tri-nucleotides with amino acids is based on hydrogen bonds one has to take the ratio of the number of bonds the amino acid can form to the number of bonds the determinating di- or trinucleotide can develop. This ratio, however, has to be considered under the aspect of already given amino acid and nucleic acid components. So hydrogen bonds of the 2'-hydroxy group should not be considered if the amino acid does not have a hydroxy or mercapto group. Amino acids, which are determined by an anticodon with a moveable uridine nucleotide, which is not in a cage-like structure, will fall through this determination scheme due to the poor tendency of uridine to form such hydrogen bonds. The foregoing figures show that, besides four to seven formed bonds, maximally one or two remaining binding possibilities—either on the amino acid or on the anticodon—exist, so that between 80 to 100% of the possible bonds are formed.

2. Strength of Interaction between Aliphatic Amino Acids and Nucleic Acids

The proposed amino acid-anticodon correlation is mainly based on hydrophobic bonds between aliphatic hydrogen atoms and aromatic π-electrons. These bonds are generally considered as being very weak or non-existing.

According to the electrostatic model the strength of a hydrogen bond depends on the degree of positive charge on the hydrogen atom and on the lone-pair or π-electron charge distribution of the atom or molecule to which this bond is formed (Kollman and Allen, 1972).

The charge of the aliphatic hydrogen atoms of an amino acid will be influenced by the charges of the amino- and carboxyl groups. Therefore a certain influence of the different modifications an amino acid undergoes during protein synthesis will exist. The free amino acid will show no tendency for such interactions due to the negative charge of its carboxyl group. But this tendency will increase for aminoacyl-AMP and aminoacyl-*t*RNA molecules with an anhydride or an ester bond. The π-electron charge distribution of the bases will not only depend on the kind of the nucleotide; it might also be influenced through the negative charge of the phosphate groups which can be neutralized by cations.

According to the stereospecificity between amino acids and anticodon-nucleotides proposed here, one should examine the conditions during the process of aminoacylation of *t*RNA. In this process one can consider the *t*RNA as an enzyme which catalyzes—with the help of the synthetase—the connection of an amino acid residue to itself. Concerning the role of enzymes in the catalysis of chemical reactions one of the most perceptive comments is probably that of Pauling (Pauling, 1948a, b): "I think that enzymes are molecules that are (according to Lipscomb, 1972, better:

... that become ...) completementary in structure to the activated complexes of the reactions that they catalyze, ...". An idea which is today already supported by a number of experimental results, showing that complementarity and strong binding has to be given in the activated complex (Lipscomb, 1972).

The process of aminoacylation is an esterification process. It is most probably a proton-catalyzed reaction, with the proton being bound to the carboxyl group (Gould, 1959). Base catalysis should cause a tautomeric equilibrium between 2'- and 3'-esters which does not occur, however (Wolfenden, Rammler and Lipmann, 1964). Therefore the interaction of such protonated amino acid derivatives with aromatic π-electron systems has to be studied, since in this case strong interactions causing complementarity should exist.

Proton magnetic resonance was chosen as measuring method. Measurements were made with a High Resolution NMR JEOL JNM-PS-100 at room temperature. Ethylglycinate hydrochloride, which was used as amino acid derivative, carbon tetrachloride, benzene, tetramethylsilane, and trichloroacetic acid were obtained from Merck, Darmstadt, W. Germany, in their purest form and used without further purification.

Ethylglycinate hydrochloride can be dissolved in carbon tetrachloride by adding trichloroacetic acid. In such acidic non-aqueous solvents a protonation of the carboxyl group of an ester occurs (Olah and White, 1967). This protonation causes an increase in the positive charge of the neighbouring methylene hydrogen atoms indicated by an increasing downfield shift of these peaks with increasing trichloroacetic acid concentration in regard to tetramethylsilane as internal standard (Fig. 18).

It was not possible to study the interaction of nucleosides and bases with ethylglycinate under these conditions since simultaneous protonation did not allow to observe the proposed interactions. Therefore benzene, which is not so easily protonated, was taken as aromatic model compound.

As was expected, the NMR signals of the methylene groups shifted upfield with increasing addition of benzene. The value of the upfield shift increases proportionally to the strenght of the preceding downfield shift (Fig. 18).

The upfield shift caused by aromatic π-electrons is usually explained through the influence of an aromatic ring current on hydrogen atoms situated above the plane of the aromatic system. This shift increases with decreasing distance of the hydrogen atom from the center of the aromatic plane (Johnson and Bovey, 1958).

The various equilibria in this solution, (e.g.: dissociation of the acid; protonation of the ester; protonation of the benzene; complex formation between benzene and protonated or unprotonated ester) make the determination of the thermodynamic data for the complex formation between

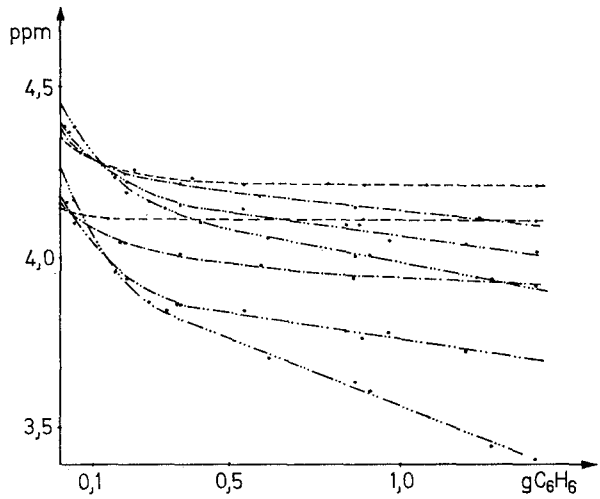

Fig. 18. Binding of the methylene groups of ethyl glycinate hydrochloride to the π-electrons of benzene depending on the protonation of the ester. A 0,2 *M* solution of ethyl glycinate hydrochloride in carbon tetrachloride was obtained by trichloroacetic acid in different molar ratios: 1:1 ------; 1:2 -·-·-·-; 1:5 -··- ··-··-; 1:10 -···-···-. Tetramethylsilane was used as internal standard. The shifts of the methylene groups of glycine obtained by addition of benzene are given in the four lower curves. The four upper ones are from the methylene group of the ethyl group

protonated ester and benzene somewhat difficult. But it is possible to consider the size of the shift already as an indication of the strength of a hydrogen bond if the anisotropy of this complex is constant and as far as hydrogen bond length and bond strength are correlated (Rabinovitch, Gilderson and Blades, 1964). The knowledge of the shift of the pure protonated acids or esters should thus allow to extrapolate the shift of the complex of the protonated amino acid with benzene from the values of Fig. 18.

Protonation of a carboxylic acid, measured at —60 °C, causes a shift of the α-hydrogen atoms of about one ppm [e.g. the methyl group of acetic acid is shifted from 2,10 ppm (Varian, 1962) to 3,20 ppm] and of an additional ppm for the oxocarbonium ion (4,35 ppm) (Olah *et al.*, 1967). Using these values, generally valid for carboxylic acids, to extrapolate the data of Fig. 18 one obtains, for the protonated amino acid ester with a downfield shift of one ppm, an upfield shift of about 6,5 ppm. The oxocarbonium ion is the intermediate of an esterification if special sterical conditions prevent the formation of a complex between the reaction partners and if stabilization of the positive charge by electron-donating groups exists (Gould, 1959), a mechanism which cannot be excluded in this case. It would result with a downfield shift of about two ppm in a quite enormous upfield shift of 13 ppm.

These values have to be compared with the usually obtained upfield shifts of one to two ppm which correspond to interaction energies of about one kcal/mole (Ronanyne and Williams, 1969), suggesting that during an esterification process the proposed bonds are strong enough to cause complementarity in an activated complex.

In regard to these results, theoretical calculations of hydrogen-π bonds should be mentioned. LCAO calculations of such a system yield interaction energies up to 12 to 16 kcal/mole with increasing positive charge of the aliphatic hydrogen atom (Hartmann and Strehl, unpublished results).

3. Evolutionary Aspects

The chemical evolution of organic substances which occurs on planets possessing a reductive atmosphere containing hydrogen, methane, and ammonia, and liquid water, leads to a mixture of different organic substances (Calvin, 1969). Amino acids, sugars, aromatic bases, porphyrins, etc. are formed in a random synthesis (Ponnamperuma, 1971; Ring, Wolman, Friedmann, and Miller, 1972).

Heat, polyphosphoric acid and organic condensing agents form polymer protein- and nucleic acid-like substances (Calvin, 1969). These polymer and monomer organic substances agglomerate in water to micelles and coacervates (Fox, 1969). Besides this structure-forming function, water also saves the biomolecules from being decomposed since it absorbs the short-waved UV-rays, hostile to life, which penetrate the reducing atmosphere. Thus water becomes the element of life.

The problem of how life began under these conditions on earth becomes the problem of how polymers began to be copied in the processes of replication and translation.

The molecular basis for the process of replication is given through the hydrogen bonds between the bases, which correlate adenine with uracil and guanine with cytosine (Eigen, 1971). This complementary nature of the nucleic acid constituents explains the autocatalytic property of these compounds to multiply themselves (Schramm, Grötsch, and Pollmann, 1962). Such a complementarity does not exist for the amino acids. Therefore one can assume that nucleic acids will have influenced the synthesis of definite proteins from the very beginning of life.

According to the interactions proposed above, a nucleic acid will be able to select specifically amino acid derivatives out of the primordial soup. Especially such derivatives will be intercalated which are able to form the above described protonated complexes. These derivatives are anhydrides with phosphoric acid, carrying a proton on the phosphate group which can form a hydrogen bond to the carboxyl group of the amino acid, as it also happens for aminoacyl-AMP in the process of aminoacylation of today.

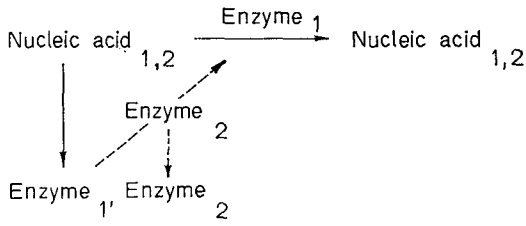

Fig. 19. The most primitive scheme of a living system

The connection of intercalated amino acid derivatives to a protein leads to a primitive translation of a nucleic acid sequence into a protein. This process might have been catalyzed by randomly synthesized proteins which possess already diverse catalytic activities (Fox and Krampitz, 1964).

There exists already experimental evidence that polynucleotides influence the condensation of different aminoacyl-AMPs in a micelle built up of randomly synthesized proteins. Depending on the experimental conditions, codon or anticodon specificity was found for single aminoacyl-AMP species (Nakashima and Fox, 1972).

During millions of years, the nucleic acids will have been changed by mutation, inducing also changes in the coded proteins. A system of this kind will multiply itself much faster if the coded protein possesses catalytic activity either on the process of nucleic acid multiplication or on the mechanism of connecting intercalated amino acid derivatives. This development will finally lead to the selection of a system with two coded enzymes, one for the process of replication and the other for the process of translation (Fig. 19).

Eigen sees the evolution of life, starting from randomly synthesized polymers, in the coupling of nucleic acid and protein synthesis in a self-reproducing Hyper-Cycle (Eigen, 1971). A nucleic acid (1) promotes the formation of a protein (I). This protein accelerates the formation of a nucleic acid (2). This second nucleic acid promotes the formation of a new protein (II) and so on, till one comes to a protein (n) which accelerates the formation of the nucleic acid (1), closing the selfreproducing Hyper-Cycle. In this system one has a lot of polymerases specific to a certain nucleic acid each of them accelerating the formation of a special nucleic acid. By reducing these polymerases to only one species and explaining the way in which a nucleic acid can promote the formation of a protein —by an intercalation mechanism and with the catalytic activity of a second protein—one can reduce the Hyper-Cycle to a very primitive system of only four components (Fig. 19).

One has to expect that the accuracy of such a primitive translation mechanism will be relatively low. Different compounds can bind between two certain nucleotides. Glycine for example, as smallest amino acid, is

able to bind between nearly all dinucleotides. It will be mainly displaced by other amino acids so that C-C as the most adapted one remains. These equilibria will have been influenced by the composition of the primordial soup, by its amino acid and nucleic acid concentrations, the existence of diverse other compounds, the ion concentrations, and the pH.

In regard to the importance of the translation mechanism for the whole cell, this primitive mechanism will have to evolve to a more efficient one. An evolution to higher accuracy would have been possible through a decrease in the number of amino acids or by the further modification of the nucleic acid, increasing the number of nucleotides. Both possibilities have great disadvantages with respect to the decrease of either the multiplicity of protein structures or of the accuracy of the replication process. Therefore the third possibility—the evolution of a new translation mechanism where the specificity is determined through more accurate interactions between macromolecules—will have occured. One has to expect that both mechanisms have produced the same proteins with the same templates, since in this case the information content of the already existing templates could be used. This leads to the present day mechanism of protein synthesis where the original nucleic acid information is transcribed two times, allowing the original interaction between amino acids and anticodons: into the *m*RNA and into the *t*RNA molecules which are filed according to the *m*RNA in the process of protein synthesis. The *t*RNA molecules serve as new templates for the amino acids.

According to this, the adaptor molecules result from a later stage of the evolution where the accuracy of the protein synthesis mechanism is changed from the interactions between monomers to the polymer level.

In the present day mechanism of protein synthesis, the specificity of the aminoacylation process is determined by the interaction between the two macromolecules *t*RNA and synthetase. But if the correlation of an amino acid to a trinucleotide is considered to depend only on these macromolecular interactions, one is surprised that a universal genetic code exists. Mutations should have allowed a certain degree of variability of the genetic code during three billion years. Since this is not the case, one is led to assume that a chemical restraint continues to operate at the present time, perhaps during the attachment of amino acids to *t*RNA (Weinstein, 1963; Woese *et al.*, 1966a).

But it is not only from evolutionary aspects that the problem of stereospecificity of the genetic code becomes connected with the tertiary structure of *t*RNA. In the two preceding chapters, helpful conclusions could have been drawn from the assumption of an amino acid-anticodon interaction in the process of aminoacylation and also indications for such a connection were derived. Therefore it should be mentioned that there exists a structural possibility for *t*RNA to adopt such a tertiary structure (Melcher, 1972).

Two interactions may guarantee the specificity and universality of the genetic code of today: the macromolecular interaction between synthetase and *t*RNA with its dominating role and high specificity and the amino acid-anticodon interaction disposable for the specificity but necessary to keep the genetic code universal.

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