Über eine quantitative Einschätzung der Woolle-Hypothese für ein Polynucleotid-System

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Crick 1, has proposed a hypothesis for the codons-anticodons base pairings in which he assumes that similar codons with only its third base different can be paired with the same anticodon. In this way Inosine, in the anticodon, can be matched with Cytidine, Adenine or Uracil, if these bases are at the end of the respective codon; Guanine, in the anticodon can be paired with Cytosine or Uracil, if these are in the third position of the codon; and Uracil, in the anticodon, can be matched with Adenine or Guanine, when the codon ends in these bases.

This hypothesis permits to reduce greatly, the total number of the transfer RNAs maintaining at the same time the code degeneracy, at the codons level, which could be necessary for the security in the exact transmission of the genetic message.

In the ordered plan of aminoacid codons given by Crick, the code degeneracy is achieved, in general, by a permutation of the third base of the codons 2; this therefore makes clear the role played by the third base in the codon-anticodons pairing. However, it is obvious that e. g. for isoleucine codification it is the third base which differentiates this codon from that of the methionine. This is a very singular fact, because methionine plays an important role, in the initiation protein chain at least in the case of B. coli. Furthermore, in the case of aminoacid which present ambiguity in the first two bases of its codons it is the third base the one which characterize them, e. g. Phenylalanine with UUU and UUG as codons and leucine codified by UUA and UUG codons.

As the nature of the third base is apparently important in many codons, it is then interesting to try to evaluate the affinity of Inosine with Cytosine, Adenine, or Uracil as well as the affinity of Uracil with Adenine or Guanine.

In an experimental model we have studied the kinetics of poly I pairing with poly C, poly A, or poly U, and as measurement of the amount of matching we utilize the hypochromism observed in the U.V. zone of the spectrum, when there occurs polynucleotide pairing forming a complex through hydrogen bonds. During these experiments similar conditions of polynucleotides and salts molarities, pH, and temperature of the samples were maintained 3–5, Polynucleotides were obtained from Calbiochem.

In Fig. 1 curves of absorbancy versus time are shown for mixtures of poly I plus poly C, poly I plus poly A and poly I plus poly U. The shape of these corresponds to a second order reaction kinetics, at least for the first part of the pairings studied, and we see that under the (similar) conditions studied, the rate constant of poly (I C) complex formation is of $1.125 \times 10^{4}$ l/mol, seg., of $0.875 \times 10^{3}$ of/mol. seg., for poly (I A) complex formation and for poly (I U) complex pairing is too low to be measured without the risk of polynucleotide hydrolysis.

![Fig. 1. Second-order plot of formation of: Poly (I C) 232 m\mu \text{C} \cdots \text{C}, Poly (I A) 252 m\mu \text{A} \cdots \text{A}, Poly (I U) 254 m\mu \text{U} \cdots \text{U}, (X^*)_{t} = O.D_{90} - O.D_{90}(X). Conditions: 0.01 M NaCl and 0.01 M NaAc. (pH 6.8) Polynucleotide mixture 1:1,4 \times 10^{-4} M. (The concentration of Polynucleotide is expressed as nucleotide residue.) Temp.: 11 ^\circ\text{C}.](image)

If, following a first approximation, we accept the validity of the Arrhenius equation for those models,
it follows that the differences of activation energies between poly (I C) formation and poly (I A) pairing is of the order of 2.155 Kcal. and between poly (I C) matching and poly (I U) formation, is so big, that in spite of changes in the standard conditions of work (sodium chloride molarities between 0.02 — 0.15 M and temperatures between 8° C—25° C), we could not appropriately estimate it. Due to the differences in the activation energies of the formation of these complex we have to accept that, if the concentration of these polynucleotides are maintained then in an infinite time only the reaction with smaller activation energy should hold. However, as the biosynthesis of protein occurs at a very fast rate (10^4 bases being paired by second 6) and in a unidirectional way in space, it could happen that for one aminoacid having different codons with the same two first bases and only the third one being different, the pairing of codon-anticodon complex would not always have the same value. In effect, if the anticodon ends in Inosine, the codon which has a third base with the greatest rate (affinity) for matching with Inosine should have the smaller probability of error and the codons which end with the base with lower affinity for Inosine should have the greater probability of mispairing. The observed pairing of poly U with poly I is very slow and as the Inosine-Uracil pair has the same hydrogen bonds as the Guanine-Uracil pair (N_H—OC_2; C_O—H_N_2), the Uracil in these pairings having a rotation of 2 1/2 A (Crick 1), we can assume (work in progress) that the Guanine-Uracil pairing even if possible, occurs at a very slowly rate and then in the aminoacids with degenerate codons ending in U or C, the matching with an anticodon finishing in a Guanine base should be safer in the case of Guanine-Cytosine pairing.

A similar situation holds for aminoacid with degenerate codon endings in Adenine or Guanine, when matching with anticodons ending in Uracil; here the trinucleotide that ends in Guanine should have greater possibilities of mispairing that those ending in Adenine. The Wobble Hypothesis and the facts described in this communication do not necessarily mean evolutionary selection of the most efficient codons and disappearance of the less safe ones, because this selection should imply also the detriment of code degeneracy at the level of DNA and mRNA, which is not convenient for a faithful transference of genetic information.

It is possible to question the extrapolation to trinucleotide, of the values of the rate constant of polynucleotide pairing, but we believe that these data give a good approximation to codon-anticodon interactions because it corresponds to pairing of stacked bases and in this sense, they are more truthful than the schematic pairing of bases only. Besides, the quantitative knowledge of the polynucleotide interactions could give a better understanding of the biological phenomenon of mutations. (Work in progress.)

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Eine neue colorimetrische Methode zur Bestimmung von Indolauxinen

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Zur Bestimmung der pflanzenphysiologisch wichtigen Indolauxine stehen verschiedene Methoden mit zahlreichen mehr oder weniger empfindlichen Abänderungen zur Verfügung 1—3.

Das hier mitgeteilte Bestimmungsverfahren basiert auf einer neuen zur Bestimmung des Tryptophans angewandten Reaktion des in β-Stellung substituierten Indols mit Fructose und Cystein in 70-proz. Schwefelsäure 4. Es zeigt ausreichende Genauigkeit und Reproduzierbarkeit, vorausgesetzt, daß das quantitativ zu bestimmende Auxin von anderen Indolderivaten (z.B. auf chromatographischem Wege) befreit wird.

Die quantitative Bestimmung des Auxins

Zur Bestimmung des Auxins sind die folgenden Reagenzlösungen erforderlich:

1 J. A. Bentley, Methods biochem. Analysis. 9, 75 [1962].
2 S. A. Gordon u. L. G. Palég, Physiol. Plantarum 10, 39 [1957].