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BIOCHEMISTRY

ON THE RÔLE OF THE NUCLEIC ACIDS IN THE BIOSYNTHESIS OF THE PEPTIDE BOND

BY

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Introduction

With reference to the statements of CASPERSSON [1, 2], BRACHET [3–6] and other authors [6–12], suggesting on divergent grounds a still undefined rôle of the nucleic acids in the biosynthesis of proteins, a working hypothesis on this biosynthesis was put forward in this laboratory about a year ago.

In order to provide some experimentally supported details about one part of our hypothesis a study was made on the reaction kinetics of the hippuric acid synthesis described by CHANTRENNE [12].

The results of our measurements and our working hypothesis as far as it is related to them are presented in this paper. Although this hypothesis has some features in common with the one recently proposed by DOUNCE [13] we are led to suggest the occurrence of rather different intermediate compounds during an essential stage of the peptide chain synthesis.

Experimental part

The model synthesis of the peptide bond described by CHANTRENNE [12] involves the use of phenyl benzoyl phosphate (P.B.P.) and glycine which, under physiological circumstances, react together up to 99 % to form hippuric acid.

All our experiments were carried out at 37° C; the reagents were dissolved in 0.1 m phosphate buffer. The sodium salt of P.B.P. was prepared as described by CHANTRENNE [12].

Under highly standardized conditions reaction velocities were measured by colorimetric determination of the acyl phosphate according to the method of LIPMANN and TUTTLE [14]. After well-determined times of incubation, 5 ml of the reaction mixture was rapidly mixed with 5 ml of 2 m hydroxylamine solution, pH 6.0 and incubated at 37° C for 90 minutes, the formation of benzoyl hydroxamic acid being completed after this period. After that 20 ml of 1.66 % $FeCl_3$ solution in 1 N HCl was added and the extinction, due to the colour of the ferric benzoyl hydroxamate, was measured at an average wave-length of 550 mμ by means of an "Engel" colorimeter.

From the extinctions measured the percentages of P.B.P. left after different times of incubation with glycine were computed and plotted in a curve from which the course of the concentration of P.B.P. with the time of incubation could easily be read.

Table I and figure 1 illustrate results of the procedure outlined above.

TABLE I

Reaction velocity at 37° C of a solution of 0.0025 m P.B.P. and 0.1 m glycine in 0.1 m phosphate buffer pH 7.39. No measurable hydrolysis of P.B.P. occurred.

Time of incubation in minutes	% of P.B.P. left	$K_{mon.} \times 10^4$ (sec.$^{-1}$)
0	100	—
5	88.1	4.2
10	77.5	4.2
15	68.3	4.2
20	60.2	4.2
25	53.1	4.2
30	47.2	4.2
35	42.1	4.1
40	37.8	4.1
45	33.9	4.0
50	30.4	4.0
55	27.1	4.0
60	24.1	4.0

Furthermore, the spontaneous hydrolysis of P.B.P. was determined separately under the same conditions as was the velocity of the peptide

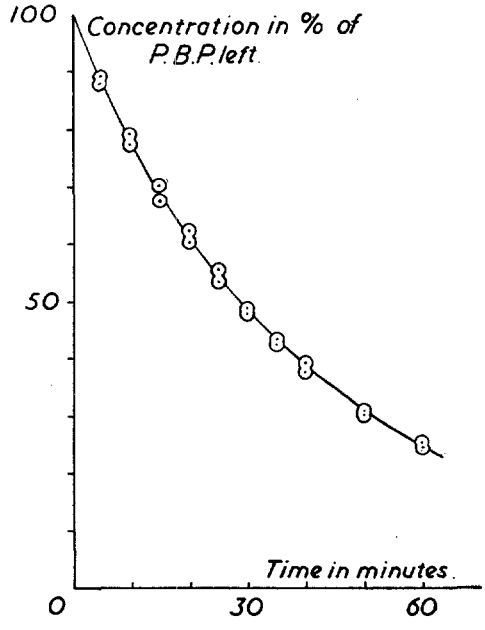


Fig. 1. Course of the concentration of P.B.P. with the time of incubation under the experimental conditions described in table I.

bond synthesis. Whenever necessary corrections for hydrolysis were made, though P.B.P. solutions appeared to be remarkably stable over the pH range from about 6.2 to 8.0.

Measurements were carried out at pH 7.39, 6.65 and 6.29 with different concentrations of P.B.P. and glycine. We proved the reaction to be first order with respect to the concentration of P.B.P. in all cases investigated. This finding is not in agreement with the data published by CHANTRENNE [12], indicating the reaction to be second order with respect to the concentration of this compound.

In addition, by variation of the pH and of the concentration of the glycine, we could prove the reaction to be also first order in the concentration of glycine and of OH^- over a pH range up to 7.4. See table II.

TABLE II

Computed and determined rate constants at pH 7.39, 6.65 and 6.29 with different concentrations of P.B.P. and glycine.

Exp. nr.	pH	Conc. P.B.P. (moles/l)	Conc. glycine (moles/l)	Pseudo first order rate constant $\times 10^5$ (sec^{-1})	
				Computed	Determined
1	7.39	0.0025	0.1	—	41
2	7.39	0.00125	0.1	—	40
3	6.65	0.00125	0.1	7.9	8.7
4	6.29	0.00125	0.1	3.5	3.6
5	7.39	0.00125	0.044	18	19

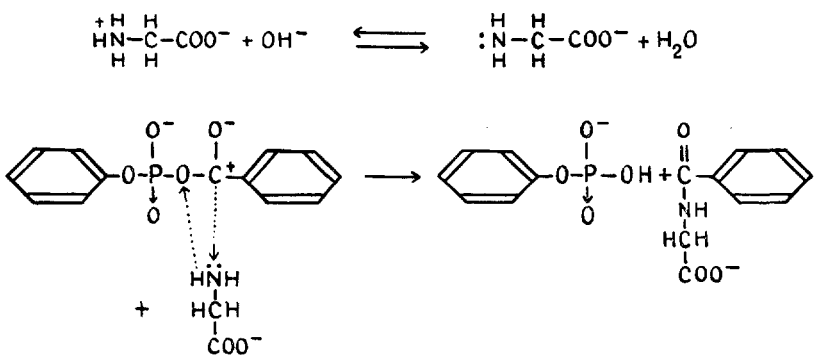
Consequently the reaction velocity, v, can be given as

$$v = K\,[P.B.P.]\,[glycine]\,[OH^-]$$

with $K = 0.7 \times 10^{10}$ ml^2 mol^{-2} sec^{-1}.

Discussion

The following reaction scheme involving nucleophillic attack of the carboxyl carbon by the free electron pair of the amino group nitrogen is in good harmony with the data described above:



It is also in agreement with the reaction scheme proposed by KOSHLAND [15] for the acylation of amino acids and amines by acetyl phosphate.

The reactivity of the acyl phosphate bond suggests that one of the functions of the nucleic acids in the biosynthesis of the peptide chain may be the forming of intermediate high-energy acyl phosphate bonds. Moreover, it seems rather likely that the nucleic acids serve as templates [13, 16, 17] determining the structure of the proteins formed. It should therefore be assumed that at least as many specific arrangements of the functional compounds (probably the nucleotides) exist in a given nucleic acid as there are specific arrangements of amino acid residues in the protein synthesized on the "mould" of this nucleic acid.

Consequently an essential intermediate compound in the biosynthesis of proteins could be a nucleic acid, acylated with amino acid residues as indicated in fig. 2. Starting from these amino acid-nucleic acid compounds poly-peptide chain synthesis would occur almost spontaneously, the course of the synthesis being of an identical chemical nature with the model synthesis of hippuric acid described above.

In order to synthesize a complete poly-peptide chain and not a number of short oligo-peptides, the peptide bond formation according to the scheme outlined in fig. 2 should proceed upwards. This means that reaction at the phosphate acyl bond should occur easily when the phosphate is acylated with a peptide, but not when a free amino group is present. It is conceivable that in the first case steric circumstances are more favourable on account of the difference in interaction of an amino group (NH_3^+) and of a peptide bond (–CO–NH–) with constituents of the nucleic acid.

At the moment only vague suggestions can be made about the mechanism for the synthesis of the acylated nucleic acids, which should also be quite selective. Acylating interaction of phosphotransacylase systems containing coenzyme A (CoA) [18–22] or similar compounds may be involved. This interaction might be relatively non-specific, the specificity of the total reaction being due to a directing influence of the nucleic acid (nucleoprotein) itself. In connection with this influence attention should be drawn to the fact that the structure of a nucleic acid template becomes more specific by every amino acid residue that is fixed upon it. Referring to some recent data [22, 23] on the structure specificity of proteins, the degree of specificity of the nucleic acid templates must still be left open to discussion. With regard to the reaction scheme outlined in fig. 2, attention should be drawn to the following aspects:

1. Carboxy-phosphate derivatives of the nucleic acids are still unknown. So are the phospho-amide derivatives suggested by DOUNCE [13] as intermediate compounds in peptide chain synthesis. However, analogous compounds are known and have been tested for their reactivity in the synthesis of the peptide bond. From data in the literature [12, 15, 24–27]

and from our own measurements it may be concluded that a number of carboxy-phosphate compounds (e.g. substituted benzoyl-phosphates, acetyl phosphate, phtalylglycyl dibenzyl phosphate) react in vitro under

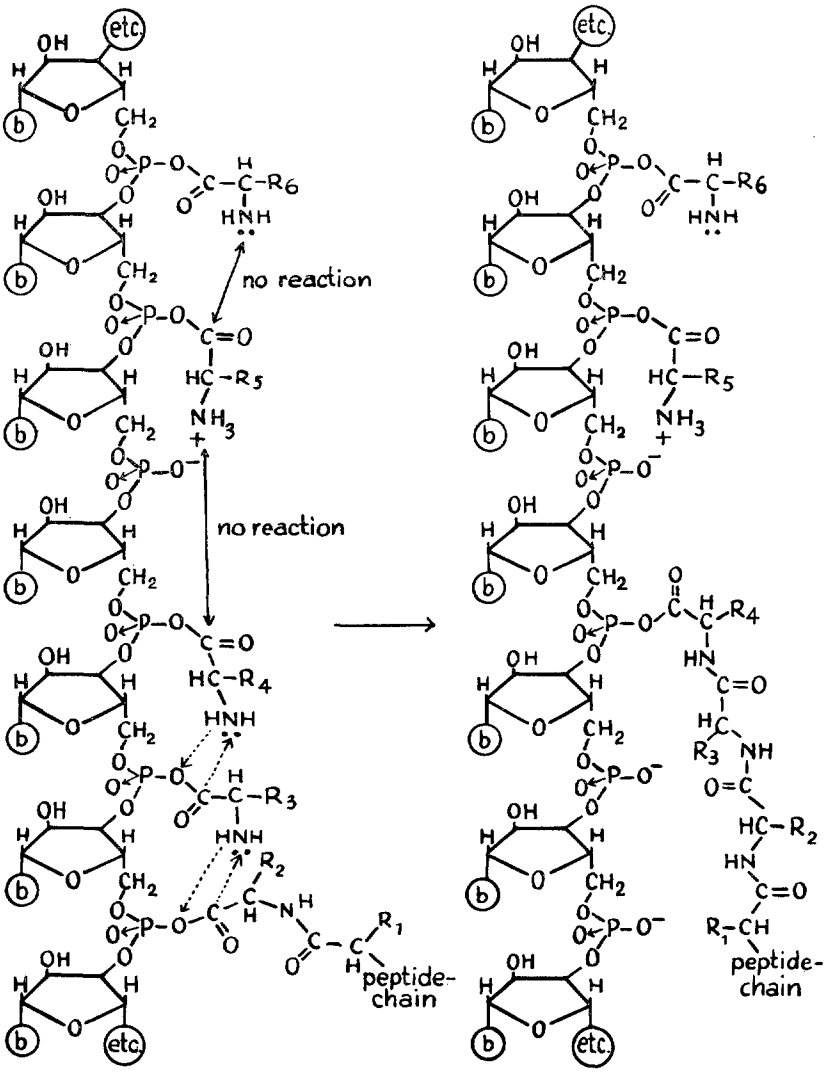


Fig. 2. Schematic outline of the synthesis of a poly-peptide chain starting from an amino acid-nucleic acid compound. b = purine and pyrimidine derivatives.

more or less physiological circumstances spontaneously to form peptide bonds. Phospho-amide compounds [27, 28, 29], however, although they may form peptide bonds, do not react under physiological circumstances.

2. In our hypothetical mechanism only one unknown labile nucleic acid compound is assumed. The necessary steric requirements for the most essential reaction during the last step in this mechanism may be

easily furnished by the nucleic acid structure itself, in contradistinction to the steric requirements for the corresponding step in the mechanism proposed by DOUNCE [13]. This fact is illustrated by Stuart models in fig. 3.

Furthermore, only a few non-specific — if any at all — enzymes regulating the reaction have to be assumed, as a spontaneous reaction may be expected.

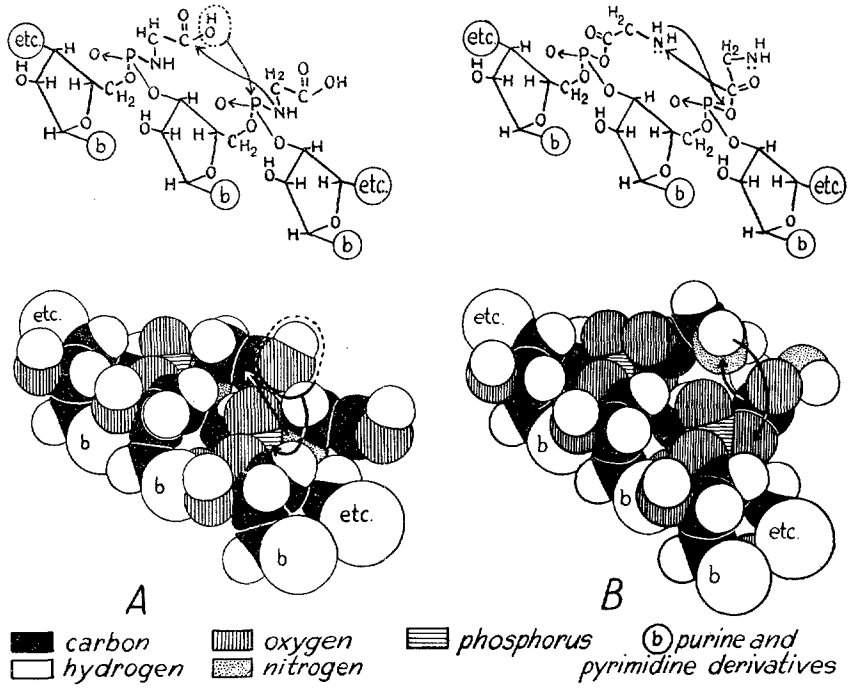


Fig. 3. Stuart models illustrating the biosynthesis of the peptide bond: A. Starting from phospho-amide compounds of the nucleic acids as suggested by DOUNCE. B. Starting from carboxy-phosphate compounds of the nucleic acids according to our hypothesis. In both models quaternary N-atoms had to be used instead of P. The glycine residues are turned into a favourable position for reaction.

3. In agreement with DOUNCE [13] a part of the specificity of the nucleic acid template is ascribed to the presence of the purine and pyrimidine derivatives.

4. Physiological interesting aspects — an interrruption of the reaction if one or more amino acids are missing on their specific places in the template and a possible correspondence in a major spacing along the fiber axis between poly-peptides and poly-nucleotides [13] — remain valid.

5. Several aspects of our reaction scheme, as for instance stability and reactivity of acylated nucleic acids and interaction of acylated compounds with nucleic acids or nucleotides can be tested experimentally.

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Summary

1. A study was made on the reaction kinetics of the model synthesis of the peptide bond as described by CHANTRENNE.

2. A reaction scheme of this synthesis is described.

3. In addition a hypothesis is presented concerning the rôle of the nucleic acids during the biosynthesis of the peptide bond. It postulates the existence of intermediate amino acid-nucleic acid compounds with carboxy-phosphate bonds and it needs a relatively small number of specific enzymes.

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