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AUTOCATALYTIC REPLICATION OF THE DIPEPTIDE BONDINGS OF A CYCLO-TETRAPEPTIDE ENFORCED BY MG^{2+}-SALINE ANHYDRIZATION

BY

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ABSTRACT

Autocatalytic replication of the dipeptide bondings of a cyclo-tetrapeptide enforced by Mg^{2+}-saline anhydrization. – Molecules of a simple, monomeric cyclo-tetrapeptide (β-Ala-Gly-β-Ala-Gly) dissolved in an anhydrizing Mg^{2+}-rich saline solution are not only stabilized but could also act as templates for their autocatalytic replication as evidenced at least by significant increases in dipeptide bondings obtained from only the homologous amino acids.

INTRODUCTION

The evolution of templates is central to the problem of the origin of life itself, defined by its main criterion, i.e. self-duplication of the primordial biomolecules. The fact that their autocatalytic reproduction occurs with the concourse of electrostatic H bonds (KAUFFMAN, 1986) emphasizes the decisive role played by bipolarity in this molecular evolution (TURIAN, 1994).

H_2O molecules were first to H bind together into self-assembled networks (see JEFFREY & SAENGER, 1994) followed by prebiologically formed amino acids covalently binded, in anhydrizing conditions, first into dipeptides themselves linked by reciprocal H-bondings into the secondary structures of polypeptides. Among them, the anti-parallely piled-up chains of β-pleated sheets appear as best habilitated for self-duplication mediated by interchains H-bondings (ORGEL, 1972, 1992). When closed upon themselves, the superposed densely H-bonded rings of such β-sheets form nanotubes (GHADIRI *et al.*, 1993) which we have recently modellized as prototypic, precellular structures or protobionts (TURIAN, 1995).

To lend experimental support to this model, we have chosen the single rings of a small cyclo-tetrapeptide on the double criterium of its commercial availability and simple composition. As working hypothesis, we have envisioned that its circular chain of only two sequentially bonded amino acids, glycine and β-alanine considered as primordial (MILLER, 1987; DE DUVE, 1991), might play a template role for its self-replication when incubated in conditions shifting the thermodynamic reaction equilibrium toward peptide bond synthesis (see LIPMANN, 1965), namely anhydrization provided by magnesium ions.

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MATERIALS AND METHODS

The cyclo-tetrapeptide (β-Ala-Gly-β-Ala-Gly) has been obtained from Bachem A.G. (Switzerland). It was solubilized either in bidistilled H_2O, plain or enriched in magnesium compounds, $MgCl_2 \cdot 6H_2O$ or MgO or the "white asbestos" chrysotile, a hydrous magnesium silicate, $Mg_3Si_2O_5$ $(OH)_4$ of the serpentine group grinded with bidistilled water in a sterilized mortar. As alternative to such solutions-suspensions, use was made of a commercial mineral water Cristalp (Saxon-Wallis, Switzerland) rich in Mg^{2+} (40 mg/l) complemented by the other cations Na^+ (20 mg/l), K^+ (1.8 mg/l), Ca^{2+} (115 mg/l) and anions such as HCO_3^- (306 mg/l) and $SO4^{+2}$, the whole at pH 6.86. Sterilized 50 ml Erlenmeyer flasks were each filled with 2.5 ml of these solutions-suspensions sterilized by filtration on Millipores (0.22 μm), each containing 1 mg/ml of the cyclopeptide without (controls) or with additional 0.05 M homologous or heterologous amino acids. The flasks were then incubated for 1 to 20 days with slow alternate shaking imitating "tide pools" in a climatized room at 25°C. After 18 days of incubation, the media had to be rehydrated to their original volume.

The amounts of preexisting peptide bonds, both from the cyclopeptide C-4P and those neosynthesized in the presence of amino acids with the concourse of the dehydration agent (Mg salts) have been assayed by the specific Lowry-Folin reagent (1951) fitted, as the biuret reaction, to assay peptide bonds. Syntheses of peptide bonds have been quantified on 50 μl at its standard extinction of E_{750nm} with reference to standard curves established with 5 to 100 μg of those 4-bonds molecules suspended in bidistilled or in saline H_2O. The optical measures could then be transcribed as μg of dipeptides (DPept in Fig. 1) synthesized from the supplied couples of amino acids in excess (Δ^+) of the 50 μg of C-4P parallely incubated alone as controls. All E values obtained have been translated into μg by reference to the standard curves and corrected in function of possible deviations, essentially due to concentration effects by progressive evaporation, of this starting 50 μg value used as 0 line to calculate the increased amounts of di (-tetra) peptides produced.

RESULTS AND DISCUSSION

Two main facts have been unraveled in our study:

(1) The need to ionically enrich the water solution of the cyclo-tetrapeptide in the presence of its homologous amino acids, glycine and β-alanine, in order to get a significant increase in amounts of peptide bonds formed. Considering Mg^{2+} ions as presumably the most efficient anhydrizers – attracting 6 H_2O dipoles – to shift the reaction equilibrium towards peptide syntheses (dipeptides, possibly tetrapeptide rings), we have tested $MgCl_2$, MgO and $Mg_3Si_2O_5$ $(OH)_4$-enriched bidistilled water and found only moderate stimulation of peptide bond formation except for that obtained with "white asbestos" (Table I).

Thinking that a more ionically equilibrated saline solution would be more efficient, we found practical to use as incubation solution a natural saline water noticeably rich in Mg^{2+} ions (40 mg/l) extracted from alpine rocks, the so-called Cristalp mineral water.

The lower and transient efficiency found with $MgCl_2$ might have been due to its relative acidifying effects during incubation. By contrast, MgO progressively hydrated into the slight alcalinizer Mg $(OH)_2$, mineralogically known as brucite, was found to be more efficient and stable in its peptide synthesizer effect. The magnesio-silicate chrysotile

TABLE I

Mg^{2+}-stimulated syntheses of peptides from the homologous amino acids (β-alanine + glycine) of the cyclo-tetrapeptide: Δ^+ µg in excess of the initially provided 50 µg template

Incubation in	Days (25°C) 1	2	4	6	12
H_2O (bidistilled)	0	0	0	0	0
H_2O + $MgCl_2 \cdot 6H_2O$*	2			5	-2
H_2O + MgO**		2		6	7
H_2O + $Mg_3Si_2O_5$ $(OH)_4$*** (asbestos)	7	12	17		
Cristalp (Mg^{2+} rich saline H_2O)	4	6	10		
Asbestos in Cristalp	7	3	6		

* 335 mg/l (24.3 mg Mg)
** 166 mg/l (24.3 mg Mg)
*** 156 mg ultrafiltered/flask

formed of bundles of flexible fibers (0.3 µm) was assayed as provider, after their grinding, of nanorods and thereby possibly playing the role of "rollers" of successive rings of the cyclopeptide according to the model proposed (see TURIAN, 1995). Surprisingly, this silicate has being the most efficient in promoting neosynthesis of dipeptide bonds from β-alanine + glycine, with a major increase after 4 days incubation in bidistilled H_2O. However, this increase was counteracted in the saline solution by a possible antagonism of the magnesio-silicate with the free Mg^{2+} of mineral water.

This first series of trials led us to generalize the use of the mineral water as solvant for the further tests reported below, concerned with: (2) The specificity of the additional peptide bonds formed from the homologous amino acids. Such an attempt was devised to ascertain the hypothesis of a template-type of autocatalytic replication provided by the homologous amino acid sequence of the cyclic peptide. In the limits of our assays, this hypothesis was confirmed as shown by the results of the comparison of efficiency of the couple β-alanine-glycine with that of half-heterologous couples such as β-alanine with either L-valine or L-leucine and glycine with the same amino acid alternative (Fig. 1).

The sharp increases of the E measured in the presence of only the homologous amino acids might not only reflect an increase in the number of dipeptide bondings (β-alanine-glycine and glycine-β-alanine), but could parallely result in a certain number of such dipeptide couples doubly closing into the yet undetected, newly replicated cyclo-tetrapeptides. In the further experiments with heterologous-homologous amino acid couples, L-valine showed to be a relatively more efficient heterologous binder than leu-

cine to either of the homologous amino acids, glycine being a better partner than β-alanine. Logically, partnership was closer between the small polar glycine and the apolar (hydrophobic) amino acids in the decreasing order of their C side-chain length. As expected, the fully heterologous couples (valine-valine, leucine-leucine) were practically inefficient (average Δ+ 2 μg). As for the "doublets", β-alanine-β-alanine and glycine-glycine,

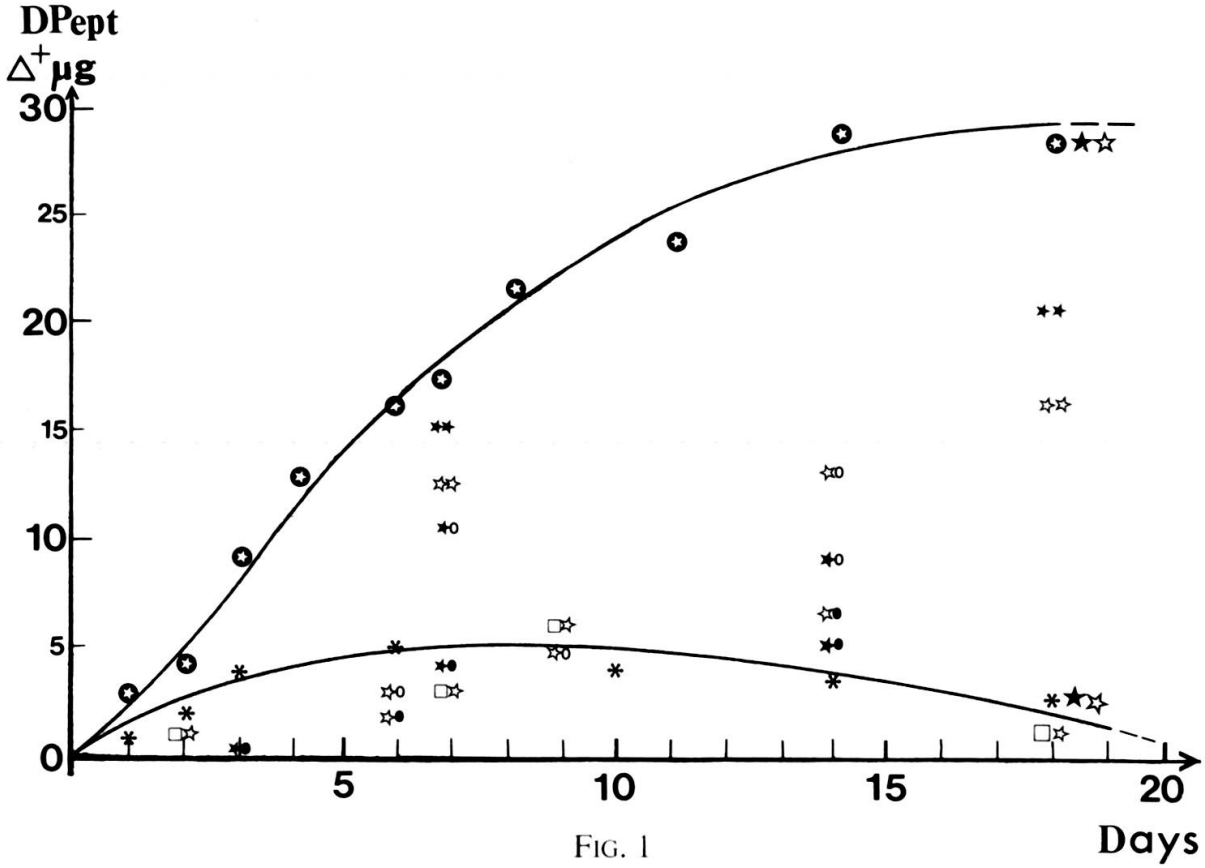

FIG. 1

Comparative efficiencies of dipeptide (DPept) bonds syntheses autocatalytically replicated on the cyclo-tetrapeptide (50 μg C-4P at 0 time) incubated at 25°C either alone for controls (deduced, not shown) or in the additional presence of couples of amino acids in distilled H_2O (*) or in saline, Mg^{2+}-rich water (☆). Optimal increases (Δ^+ μg, up to 60% in 14 days) of peptide bonding only produced by C-4P homologous β-alanine (★) + glycine (☆) in the saline solution, but sharply counteracted by α-L-alanine (□) substituted to its β-isoform. Doublets of either β-alanine or glycine were relatively effective (β-alanine especially) while replacement of β-alanine by either L-valine (○) or L-leucine (●) or of glycine by the same heterologous amino acids resulted in all diversely low Δ^+ μg dipeptide bonds syntheses.

they showed a relatively high degree of efficiency. The use of the α-"isoform" of L-alanine in association with glycine sharply lowered the efficiency of peptide bond synthesis (Fig. 1).

β-alanine is not one of the standard 20 amino acids of proteins even though it is a primordial product of the Miller-Urey type of *in vitro* sparking synthesis and component of carbonaceous meteorites (MILLER, 1987). However, it appears that it is through its linear framework configuration (-CH_2-CH_2-) enforcing attractive, hydrophobic forces that it has, in partnership with glycine, presumably conferred to the whole C-4P its unexpected high template efficiency. This would explain the inefficient replacement of

linear β-alanine by the branched α-alanine. Also would be explained the fact that glycine, with one -CH_2-, is an optimal partner for β-alanine for their dipeptidization while both branched 4C-valine and, especially, 5C-leucine are ineffective; by contrast polar 2C-glycine accepts more effectively the substitution of 3C-β-alanine by either apolarly branched amino acids.

In the saline solution, the C-4P then appeared not only to act as a template on which β-alanine and glycine are probably twice guided by hydrogen bonds to line up but also catalyze peptide binding into two dipeptides presumably prone to cyclize by template directed ligation (4th peptide closure bond of C-4P). Two C-4P ring molecules would thus become available to carry on further autocatalytic replication leading by their basipetal piling-up to the protobiontic nanotubes recently postulated. Each of its rings can thus be considered as one-unit of replication contrarily to the more sophisticated model proposed (TURIAN, 1995) involving as reproduction unit two antiparallel peptide rings endowed with oppositely charged hydrophilic amino acids, e.g. +lysine and -glutamic acid. Such doublets of peptide rings alternating those hydrophilic ones with the hydrophobic amino acids could naturally encode more information than the minimal one (1 bit) of our C-4P which, however, could drive an evolutionary potential following a possible opening of its ring to incorporate other amino acids among which electrically charged ones.

Some types of autocatalytic duplication have already been discussed by DYSON (1982) and KAUFFMAN (1993). They were first illustrated by synthesis of a linear pentapeptide prone to replicate *in vitro* (CALVIN, 1969). Closer to the cyclic type are the polypeptides organized in β-sheets, most noticeably those alternating hydrophilic and hydrophobic chains as proposed by ORGEL (1972) and BRACK & ORGEL (1975; see also CAIRNS-SMITH, 1982).

A cyclo-tetrapeptide as simple as it might have been, already fortuitously assembled in prebiological time around a brucite or asbestos-type of Mg-rich nanocrystal (see TURIAN, 1995) can now be synthesized in vitro by skilfull biochemists. Anyway, it might now provide a useful model for pregenetical "life" when we consider its templating ability to order additional specific dipeptide sequences from its homologous amino acids. If such sequences of the 4 amino acids could close on themselves by a fourth peptide bounding this would arise the interesting problem of the possible stacking of such tetrapeptides into a nanocolumn of the nanotube type produced *in vitro* by GHADIRI *et al.* (1993). This templating process would primarily involve the intensive cooperation of dipolar H-bonds between the NH^+ or the CO^- of the initial ring to interconnect with the complementary CO^- and NH^+ alternating on the second antiparallel ring.

Further experimentations will be necessary to substantiate our preliminary results on irrefutable grounds. Nonetheless, the evidence available to date appears compelling enough to warrant some hypothesis for take over of the pregenetical information provided by cyclopeptide templates.

According to a newly elaborated model-system (TURIAN, 1996) such first takeover of primordial amino acid coding sequences might have been insured by prenucleic acids born from "archetypal" doublets of nucleobases transitorily trapping the amino acids by

specific weak bondings before being strongly stowed by phosphoamide (N-P) bonds to the pyrophosphate sequences of elongating polyphosphate chains.

The evolution from prebiosis to life would thus have involved a double takeover, the first, from the *mono*molecular (amino acid) unit of the coding singlets (1 letter or bite) of the peptide system to the *bi*molecular (nucleobase-phosphate) unit of the coding doublets (2 base letters) of the prenucleic acid system, the second, from the bi- to the *tri*molecular (base-sugar-phosphate) unit of the coding triplets (3 base letters or bytes) of the "modern" ribo-deoxyribonucleic acids.

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RÉSUMÉ

Les molécules d'un simple et monomérique cyclo-tétrapeptide, dissoutes dans une solution saline anhydrisante riche en Mg^{2+}, ne sont pas seulement stabilisées mais peuvent aussi agir comme des moules pour leur réplication autocatalytique ainsi que démontré par une augmentation significative des ponts dipeptidiques à partir et seulement de ses amino acides homologues.

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