Photochemical Synthesis of Amino Acids From Paraformaldehyde Catalysed by Inorganic Agents[1]

The photochemical synthesis of amino acids from paraformaldehyde and nitrates in the presence of $FeCl_3$, or involving nitrogen fixation in the presence of colloidal molybdenum oxide, was first shown by BAHADUR et al.[2,3]. The peptide formation in the above systems was observed by BAHADUR and RANGANAYAKI[4]. The present study deals with the action of different inorganic catalysts on these systems. The results confirm that the photochemical synthesis of amino acids occurs either in the presence or in the absence of a fixed nitrogen inorganic source, and show that both the type of amino acid produced and the rate of its formation are strongly dependent on the catalyst employed.

Materials and methods. 100 ml of aqueous mixtures containing 3% paraformaldehyde, an inorganic catalyst such as $CuSO_4$ (0.1 or 0.05%), $FeCl_3$ (0.1 or 0.05%), $NiCO_3$ (0.1 or 0.05%), $CoCl_2$ (0.05%), an inorganic source of nitrogen such as ammonium carbaminate (1%), KNO_3 (1%), NH_4NO_3 (1%), or no fixed nitrogen (atmospheric source) Na_2S as a possible sulphur source, were contained in 500 ml Erlenmeyer flasks, cotton plugged and sterilized in an autoclave at 120 °C for 20 min and then exposed to a 500 W tungsten light source at a distance of about 50 cm; the control flasks were covered with thick black paper. Samples were taken under sterile conditions from time to time. The maximum irradiation time was 30 days. The test for amino acid synthesis was carried out by circular paper chromatography[2,3]. The quantitative assay of amino acid formation was limited to the comparative

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[3] K. BAHADUR, S. RANGANAYAKI, and L. SANTAMARIA, Nature *182*, 1668 (1958).

[4] K. BAHADUR and S. RANGANAYAKI, Izv. Akad. Nauk, USSR *11*, 1361 (1958).

Table I. Mixture composition: 100 ml distilled H_2O; 3 g Paraformaldehyde; 0.1 g $CuSO_4$; 2 g NH_4-carbaminate; 1 g Na_2S (a possible sulphur source). Irradiation: 500 W tungsten lamp.

Irradiation time h	Amino acids synthesized	Index of photochemical synthesis yield
0	None	None
96	Lysine	++++
168	Asparagine Valine	++ +
336	Asparagine Lysine Valine	+++ +++ +
504	Asparagine Alanine	++ +++
744	Asparagine	++++

Table II. General picture of results

Catalyst	Nitrogen source	Amino acids appearing first	Prevailing amino acids after 20 days	Prevailing amino acids after 30 days
$CuSO_4$ 0.05 and 0.1%	NH_4-carbaminate	Aspartic acid (++)	Lysine (++) Glutamic acid (++)	
$CuSO_4$ 0.05 and 0.1%	NH_4NO_3	Leucine (+)	Histidine (+) Valine (+)	
$CuSO_4$ 0.1% + Na_2S 1%	NH_4-carbaminate	Lysine (++) Asparagine (++)	Lysine (+++) Asparagine (++++)	
$CuSO_4$ 0.1% + Na_2S 1%	KNO_3	Asparagine (+)	Asparagine (++) Non-identified (+)	
$FeCl_3$ 0.05 and 0.1%	NH_4-carbaminate	Asparagine (+)	Valine (+)	Valine (++) Alanine (++)
$FeCl_3$ 0.1% + Na_2S 1%	NH_4-carbaminate	Asparagine (++) Valine (+)	Lysine (+) Asparagine (+)	Lysine (+++)
$CoCl_2$ 0.05%	Air	Glycine (+) Asparagine (+)	Proline (+) Alanine (+)	
$CoCl_2$ 0.05%	NH_4-carbaminate	Glycine (+) Alanine (++)	Lysine (++++)	
$CoCl_2$ 0.05% + Na_2S 1%	NH_4-carbaminate	Asparagine (+)	Alanine (++++)	
$CoCl_2$ 0.05% + Na_2S 1%	Air		Lysine (+++)	
$CoCl_2$ 0.05% + $FeCl_3$ 0.05%	Air	Glycine (+) Alanine (+)	Glycine (++) Alanine (+++)	Glycine (++) Alanine (+++)

Carbon source: $(HCHO)_n$. Energy source: 500 W tungsten lamp at about 50 cm.

analysis of the intensity of ninhydrine rings obtained with amino acid standards of 10–20 μg/ml, referred to as +. At the end of each experiment the sterile conditions of the mixtures were controlled by agar plate tests either for bacteria or for fungi.

Results. Of the catalysts examined, $CuSO_4$ is the most active in producing different amino acids both at 0.05 and 0.1%, especially when nitrogen is supplied by an inorganic source. This synthesis, as well as in the presence of the other catalysts, is characterized by the appearance and disappearance of definite amino acids during the irradiation time with the prevalence of some amino acids. An example of such behaviour is reported in Table I.

The further results with $CuSO_4$, with $FeCl_3$ at 0.05 and 0.1% (also an active catalyst), and with $CoCl_2$ (a good catalyst at 0.05%), are summarized in Table II.

An attempt to potentiate the yield of photochemical synthesis of amino acids has been successful: mixing $CoCl_2$ 0.05% and $FeCl_3$ 0.05% in mixtures where air was the nitrogen source. Here, only glycine and alanine are formed at the beginning and last up to 30 days of light exposure. $NiCO_3$ is practically inactive.

The non-identified ninhydrin rings were presumably due to peptide formation. The tests on the samples kept in the dark showed formation of amino acids detectable in very faint rings with Rf values different from those of photochemically synthesized amino acids.

The mechanism of formation and transformation of amino acids following photocatalysis is not as yet known. Accordingly, it is difficult to understand the prevailing formation of some particular amino acid by the action of specific catalysts. The sterility, controlled carefully throughout the experiments, is a clear indication that formation and transformation of amino acids can occur under abiogenic conditions, as was observed also by MILLER[5] using CH_4 and NH_3 in a cycled system catalysed by electric discharge, and by CULTRERA and FERRARI[6] from glucides and organic acids excited by UV-light. The importance of such a type of photochemical synthesis has been further underscored by recent observations by BAHADUR et al.[7], confirmed by BRIGGS[8], demonstrating that the action of sunlight or of artificial light on sterilized solutions containing mixtures of amino acids and organic catalysts can bring about the formation of units having properties of growth, division and metabolic activity, henceforth called *Jeewanu* (which in Sanskrit means particles of life)[9].

Riassunto. Viene confermato che la sintesi fotochimica degli aminoacidi da paraformaldeide catalizzata da composti inorganici avviene sia in presenza sia in assenza di una sorgente inorganica di azoto fissato (aria). Inoltre viene dimostrato che i tipi di aminoacidi prodotti come la velocità di formazione dipendono dal catalizzatore usato.

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Studies on the Metabolic Fate of the ^{14}C-Labeled Methyl Group of a Methylhydrazine Derivative in P815 Mouse Leukemia

Experimental and clinical evaluation of the cytotoxic agent 1-methyl-2-*p*-(isopropylcarbamoyl)benzylhydrazine hydrochloride (MBH) (NSC 77213) evoked an interest in its reaction mechanism and metabolism. The coincidence of cytotoxic[1], carcinogenic[2], and teratogenic effects[3] revealed a probable attack on the genetic material of the cell, and the breakdown in vivo of DNA by MBH[4] stimulated the search in the same direction. Our interest in this compound was increased by our observation that the terminal N-methyl group was labile[5] and by the considerable interest and speculation as to the biological significance of the normally occurring methylation of RNA and DNA in many species.

The aim of this investigation was to compare the potencies and limits of the formate pool of the mammalian cells in vivo with that of the C_1-unit derived from the N-methyl group of MBH, and to search for methylated and unmethylated purine bases in the urine of P815 leukemic mice treated either with ^{14}C-MBH or ^{14}C-Na formate.

Materials and methods. The two ^{14}C-labeled compounds, MBH (labeled in the terminal N-methyl group) and Na formate, showed a specific activity of 17.0 μc/mg and 74 μc/mg, respectively[6]. Both substances were dissolved in 0.9% saline. 2 series of 30 BDF_1 mice each, ranging in weight from 20–25 g, were inoculated intraperitoneally with 10 million P815 leukemic cells on day 0. On day 6, a group of 10 mice from each series was injected i.p. with 33.33 mg/kg (0.129 mM/kg) of ^{14}C-MBH and 1.47 mg/kg (0.0216 mM/kg) of ^{14}C-Na formate, respectively. The urine of these mice was collected in ice-cooled flasks over a 24 h period. The remaining 20 mice from each series were injected with the same doses of either ^{14}C-MBH or ^{14}C-Na formate on day 7. Their urine was collected 5 h

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