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Abiogenesis Leading to Biopoesis

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Introduction

As protein is the basic building material of all the living system of our earth, the abiogenesis of protein is one of the most important aspects of biopoesis. However protein formation starts with the search for the process under which the amino acids are formed abiogenically. Several processes have been discovered which form amino acids under abiogenic conditions. The formation of amino acids was observed for the first time by Loeb in 1913[1] who reported the formation of amino acids as glycine and alanine by passing silent electric discharge in a mixture of formaldehyde, ammonia and water. Amino acids have been synthesised by passing electric discharge in a mixture of gases by Miller[2]. The photochemical formation of natural amino acids was observed by Bahadur[3, 4, 5] in sterilised aqueous mixtures containing organic substances and inorganic catalysts. Hasselstrom exposed aqueous solution of ammonium acetate and observed the formation glycine and aspartic acid[6]. The synthesis of amino acids and other compounds of biological interest have been done using isocyanates[7], energy from ultra violet rays[8] to x-rays radiations[9] and other sources of energies have also been used for this abiogenesis.

The next important step in abiogenesis was the formation of peptide linkage. Fox[10] synthesised peptides by heating the mixtures of amino acids to 180°C for a few hours. Akabori[11] synthesised peptide by exposing aqueous mixture of amino acids to ultra-violet light. Terenin[12] suggested the possibility of effecting the reactions needing quanta of large amount of energy by radiations of shorter quanta of energy if the substrate molecules are absorbed on solid substances. Bernal[13] reported the formation of peptides by the radiations available on the earth in the mud. Bahadur and Ranganayaki in 1958 observed the formation of peptides in aqueous mixture of amino acids containing colloids of iron and molybdenum oxides as catalysts on irradiation with sunlight or artificial light from an electric bulb[14]. Perti and Pathak[15] observed the formation of peptides in aqueous mixtures using visible light and ultra-violet light in presence of inorganic catalysts. Briggs confirmed the observation of photochemical formation of peptides[16]. The synthesis of peptides in aqueous mixtures using hydrogen cyanide as the dehydrating agent has been observed by Lowe[17].

The photochemical formation of peptides in aqueous mixtures is interesting and becomes important because, put together with the observation of photochemical formation of amino acids, it is quite probable that amino acids were first formed in the oceans of the primitive earth and then these were subsequently utilized for the formation of peptides in almost the same environment or on prolonged exposure. These photochemically formed peptides have been examined for enzymic activity. The results show enzymic function as phosphatase activity.

Bahadur[18] suggested that the formation of amino acids and peptides took place in aqueous environment in the prebiological era and solar radiation and visible light played an important role in the syntheses. Then on further molecular evolution proteins were formed. This theory regarding the origin of life on the earth has been developed recently[19, 20].

In the present communication an attempt has been made to study the formation of amino acids, peptides, organic acids, sugars and enzyme activity in sterilised aqueous mixture containing glycine, methionine, aspartic acid cystein and anthracene as catalysts by exposing these mixtures to artificial light.

Experimental

A set of two mixtures in sigcol flat bottom flask of 250 ml. capacity was prepared in glass distilled water. The contents of the mixtures were as follows :

Glycine .. ..0·05 gm.
Aspartic acid..0·05 gm.
Methionine ..0·05 gm.
Cystein HCl ..0·05 gm.

and Anthracene 0·02 gm. as catalyst in 100 ml. of water.

TABLE 1

Mixture of Uncovered Flask (Light)

S.No.	Rf Values in Phenol : Water 80 : 20	Rf Values in Butanol : Acetic acid : Water 120 : 30 : 50	Identification	Remark
Microstructures :				
Hydrolysed	0·16	0·14	Cystein	Yellow
	0·15	0·28	Aspartic acid	Pinkish purple
	0·32	0·26	Serine	Purple
	0·52	0·27	Arginine	Purple
	0·58	0·34	Alanine	Purple
Unhydrolysed	0·51	0·27	Arginine	Purple
	0·61	0·81	Peptide	Purple
	0·31	0·71	Peptide	Purple faint
	0·28	0·69	Peptide	Purple faint
Environmental Medium :				
Unhydrolysed	0·16	0·14	Cystein	Yellow
	0·75	0·58	Peptide	Purple
	0·52	0·59	Peptide	Purple
Hydrolysed	0·16	0·14	Cystein	Yellow
	0·15	0·28	Aspartic acid	Purple (pinkish)
	0·30	0·35	Glutamic acid	Purple
	0·52	0·27	Arginine	Purple
	0·41	0·31	Glycine	Purple
	0·81	0·70	Methionine	Purple
	0·61	0·52	?	Purple

The flasks containing above mixtures were cotton plugged with surgical cotton, and sterilised at 15 lbs. pressure for 30 minutes in an autoclave. The mouth of the flasks was sealed with cello-adhesive tape after wrapping with polythene paper. One of the two fllasks was covered with thick black cloth and the other remained as such. The covered and uncovered flask was kept for exposure to artificial light under 1000 watt electric bulb. The temperature during the period of exposure varied from 18° to 30°C. After 560 hours of irradiation to artificial light, the mixtures of covered and uncovered flask were examined under the oil immersion microscope (D. R. P. Leitz wetzlar microscope) using aseptic conditions and no bacterial growth was observed. The sterility of the exposed mixtures was also checked by the culture-count method. Few drops of the exposed mixtures to be tested were introduced over the agar nutrient in the petridishes under aseptic conditions and kept at 35° in the incubator for three to four days. The mixtures did not show any bacterial growth during the exposure. These mixtures were analysed for their microstructures and environmental medium after separating them by the help of ultra centrifuge, each was analysed for hydrolysed and non-hydrolysed nitrogenous constituents by two way paper chromatographic technique, employing phenol-water (80 : 20) and butanol—acetic acid-water (120 : 30 : 50) as two running solvents. Hydrolysis was done with 10N—HCl in sealed, hard neutral glass ampules, kept in boiling water for 24 hours. The hydrolysed samples were dried and neutralised simultaneously in vacuum desiccator in presence of fused NaOH. Whatmann No. 1 chromatographic papers were used. Spots of amino acids and peptides were developed by spraying 0·2% ninhydrin solution in acetone over the dried chromatograms, spots of organic acids by 1% bromophenol blue. Spots of sugars were developed by ammoniacal silver nitrate. The Rf values change due to many factors such as change of solvent and also due to the degree of hydration of the same solvent.

TABLE 2

Mixture of Uncovered Flask (Dark)

S.No.	Rf Values in Phenol : Water 80 : 20	Rf Values in Butanol : Acetic acid : Water 120 : 30 : 50	Identification With 0·2% ninhydrin in acetone	Remark
Microstructures :				
Unhydrolysed	0·16	0·14	Cystein	Yellow
	0·18	0·21	Peptide	Purple
	0·43	0·35	Peptide	Purple
Hydrolysed	0·15	0·28	Aspartic acid	Pinkish purple
	0·81	0·70	Methionine	Purple
	0·16	0·14	Cystein	Yellow
	0·41	0·31	Glycine	Purple
	0·51	0·27	Arginine	Purple
	0·56	0·73	?	Purple
Environmental Medium :				
Unhydrolysed	0·16	0·14	Cystein	Yellow
	0·15	0·28	Aspartic acid	Pinkish purple
	0·31	0·39	Aleptide	Purple
Hydrolysed	0·16	0·14	Cystein	Yellow
	0·15	0·28	Aspartic acid	Pinkish purple
	0·81	0·69	Methionine	Purple
	0·41	0·31	Alycine	Purple
	0·51	0·27	Arginine	Purple

Therefore the Rf values of known, amino acids, organic acids and sugars were occasionally determined and the Rf values of unknown amino acids, acids and sugars were compared with those of known ones. The two running solvents were used in all the experiments for identification. This was accompanied by two dimensional chromatography.

TABLE 3
Mixture of Uncovered Flask (Light)

S.No.	Rf Values in		Identification	Remark
	Phenol : Water 80 : 20	Butanol : Acetic acid : H_2O 120 : 30 : 50	With 0·2% Bromophenol Blue	
Microstructures :				
Unhydrolysed	No spot	No spot	—	—
Hydrolysed	0·51	0·42	?	Yellowish
	0·32	0·21	Benzoic acid	Yellowish
Environmental Medium :				
Unhydrolysed	0·32	0·21	Benzoic acid	Yellow
	0·51	0·42	?	Yellow
	0·24	0·21	?	Yellow
Hydrolysed	0·78	0·62	Myristic acid	Yellow
	0·36	0·83	Oxalic acid	Yellow
	0·24	0·24	?	Yellow

Estimation of phosphatase activity :

The phosphatase activity was estimated colorimetrically employing the Klett Summerson photo-electric colorimeter for the measurement of intensity of colour produced using suitable filter. The analysis is based upon the measurement of the quantity of light absorbed by a coloured solution. The dilution of the solution was adjusted so as to hold Beer's Law, i.e. the scale reading was directly proportional to the concentration of phosphate ions in the mixture. To avoid the frequent change in potential of the current supply the voltage stabiliser was used.

The results of the chromatographic analysis of each flask are recorded in tables 1 to 7.

One more identical mixture, as described in the experimental portion of this paper, was prepared. It was sterilised, cooled, covered with four folds of thick black cloth and stored in a lead chamber the walls of which were made

TABLE 4
Mixture of Covered Flask (Dark)

S.No.	Rf Values in		Identification	Remark
	Phenol : Water 80 : 20	Butanol : Acetic acid : H_2O 120 : 30 : 50	With 0·2% Bromophenol Blue	
Microstructures :				
Unhydrolysed	No spot	No spot	—	—
Hydrolysed	0·32	0·21	Benzoic acid	Yellow
	0·38	0·35	?	Yellow
Environmental Medium :				
Unhydrolysed	0·32	0·21	Benzoic acid	Yellow
	0·14	0·40	?	Yellow
Hydrolysed	0·36	0·83	Oxalic acid	Yellow
	0·78	0·62	Myristic acid	Yellow

of 2·54 cm. thick sheets of solid lead. This mixture was analysed at the end of the experiment and was found to contain only the added amino acids. No other spot of amino acids, peptide, sugar or organic acid was detected in this mixture. There was no appearance of any microstructures in this mixture and the mixture had no phosphatase activity. Thus this control remained unchanged during the period of experiment.

TABLE 5
Mixture of Uncovered Flask (Light)

S.No.	Rf Values in		Identification	Remark
	Phenol : H_2O	Butanol : Acetic acid : H_2O	With Ammoniacal Silver Nitrate	
Microstructures :				
Unhydrolysed	0·53	0·31	Mannose	White
	0·24	0·52	?	White
	0·56	0·22	Sucrose	White
Hydrolysed	0·53	0·31	Mannose	White
	0·52	0·28	Glucose	White
	0·26	0·50	?	White
Environmental Medium :				
Unhydrolysed	0·53	0·31	Mannose	White
	0·26	0·52	?	White
Hydrolysed	0·53	0·31	Mannose	White
	0·52	0·28	Glucose	White
	0·36	0·56	?	White

Discussion

The chromatographic analysis of irradiated aqueous mixtures containing glycine, aspartic acid, methionine, and cystein and anthracene as catalyst indicated the presence of original amino acids added in the mixture together with a few peptide spots. This mixture on hydrolysis showed the presence of all the original amino acids and also gave newer spots which were identified as, arginine, alanine, and seriene. Similar mixture kept in dark showed the presence of all the originally added amino acids together with a few peptide spots. The mixture on hydrolysis gave the spots of the added amino acids and also of arginine, alanine and seriene as in exposed mixture. In general it has been observed that there were a few spots in unhydrolysed samples, but the same sample on hydrolysis indicated several spots which revealed the constituent amino acid of those peptides which did not appear over the chromatogram of the unhydrolysed sample. The complete identical mixtures kept in dark furnished peptide spots but these were lesser in number than those of the exposed mixture. It has been observed by Bahadur and co-worker[19] in their studies of the photochemical formation of amino acids and peptides that the control identical mixtures kept in dark also show some amino acids and peptide synthesis. They further observed that similar mixtures kept in lead chamber remained unchanged. The fact is that radiations which penetrated through the thick black cloth and reached the mixture kept in dark were also effective in the synthesis of some of these products. In the above lead chamber which had one inch thick wall of solid lead, all the radiations including cosmic radiations were cut off and no change in the mixture was effected. Microstructures were observed in the mixtures after exposure. Similar micro structures but lesser in number were also observed in the identical mixture kept in dark.

TABLE 6

Mixture of Covered Flask (Dark)

S.No.	Rf Values in		Identification	Remark
	Phenol : H_2O	Butanol : Acetic acid : H_2O	With Ammoniacal Silver Nitrate	
Microstructures :				
Unhydrolysed	0·53	0·31	Mannose	White
	0·56	0·22	Sucrose	White
Hydrolysed	0·53	0·31	Mannose	White
	0·52	0·28	Glucose	White
	0·36	0·56	?	White
Environmental Medium :				
Unhydrolysed	0·53	0·31	Mannose	White
	0·32	0·48	?	White
Hydrolysed	0·53	0·31	Mannose	White
	0·36	0·56	?	White

It is interesting to note that the microstructures of the mixtures exposed to light and kept in dark did not show the presence of any organic acid. The micro structures on hydrolysis showed the presence of myristic acid, oxalic acid and benzoic acid. The environmental media of these mixtures also showed the presence of these organic acids after hydrolysis. A few spots of some organic acids were recorded but these could not be identified in both the cases.

The micro structures formed in the exposed and unexposed mixtures showed the presence of sugars as mannose and sucrose. These on hydrolysis gave some more spots of sugars

TABLE 7

Phosphatase Activity in the Mixture Examined by Klett Summerson Photoelectric Colorimeter

S.No.	Boiled				Unboiled			
	0 min.	20 min.	40 min.	80 min.	0 min.	20 min.	40 min.	80 min.
Spontaneous (Control)	12	13	15	15	15	14	13	13
Uncovered (Light)	14	16	20	20	9	12	16	16
Covered (Dark)	30	32	35	35	16	15	15	15

along with those present in unhydrolysed ones. A similar observation was made in the environmental medium of the exposed and unexposed mixtures. More of the sugar was formed in the environmental medium in comparison with that of micro structures synthesised in the exposed mixture. However a larger quantity of sugar was present in the microstructures than in the environmental medium of unexposed mixtures.

The exposed and unexposed mixtures were examined for enzymic activity and more of the phosphatase activity was observed in the unexposed mixtures. This phosphatase activity was found to be destroyed on boiling the exposed and unexposed mixtures at 100°C for five minutes.

The greater enzymic activity in the mixtures kept in dark may be due to the stability of the peptides synthesised in these mixtures because of the mild source of energy affecting these mixtures and there is less of the thermodynamical possibility of the decomposition of these peptides in the mixture than in the similar exposed mixtures.

Summary

The mixtures containing amino acids as, glycine methionine, aspartic acid and cystein hydrochloride and anthracene as catalyst, on exposure to artificial light indicated the presence of some new amino acids other than originally added, organic acids, sugars and enzymic activity. The similar mixture kept in dark also indicated the presence of new amino acids, organic acids and sugars, but lesser in number as compared with exposed (light) mixtures. However enzymic activity was more pronounced in dark (unexposed) mixture than in light one, and in a lead chamber only the added amino acids were present.

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