Strong Peroxidase-like activity in Synthetic Molecular Associations

By

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Peroxidase is widely distributed in plants(1,2) and is present in some animal tissues. Its distribution in microorganisms has not been studied in detail. Though the presence of catalase-like activity has been suggested as essential for ecbions by many scientists, the peroxidase-like activity has not been given much thought. Here we report the peroxidase-like activity of a type of syntheti molecular associations.

During the last decade scientists working in the field of origin of life have been investigating the properties of groups of molecules of different substances when they come together, remain together and function in harmony, for it is quite probable that some such molecular association formed the first selfperpetuating system which could be called the earliest living thing. This resuled in the synthesis of a number of molecular associations (3,4,5,6,7).

The study of the factors responsible for their formation, of the properties of the group of molecules and of the chemical transformations made possible by such arrangements is in progress in various laboratories. Among these microstructures those which are formed from simple compounds, as in mixtures containing formaldehyde, biological minerals, ammonium, phosphate and metallic ions to begin with and have amino acids, peptides, sugars, purines bases are of great interest because these help in tracing the path of abiogenesis of the biochemicals as well as the arrangement in specffic molecular associations which may be called protocells (5,8,9). The search for the enzyme-like activities in artificially made microstructures is one of the most important aspect of the problem of origin of life.

In a mixture of formaldehyde, ammonium molybdate, diammonium hydrogen phosphate and biologial minerals a type of particles which have amino acids, peptides, sugars and material with catalase-like activity are formed. These

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particles have boundary wall and intricate internal structures and their ~~their~~ chemical nature and morphology will be published else where. This paper reports the peroxidase-like activity of these particles.

It has been suggested by many scientists thatperhaps life originated when the earth's atmosphere was devoid of oxygen (10,11),These earliest cells utilised the organic substances already present in their environment as their source of carbon nutrition. This makes the presence of catalase like activity as one of the essentials if a system was to act as a selfperpetuating system in presence of organic carbon(12,13).

As appreciable catalase-like activity has been observed in these particles we tested for peroxidase-like activity in them and it was found that these particles have strong peroxidase-like activity.

Experimental:

To prepare the particles 2ml. of 18% (w/v) ammonium molybdate solution, 1.4 ml.of 12% (w/v) diammonium hydrogen phosphate solution, 2 ml. of mineral solution* and 1 ml. of distilled water were taken in a test-tube . The mixture was cotton plugged, sterilised at 5# lb.pressure for 30 min., incubated for 24 hr. at 18° C. and sterilised again. To this 2 ml. of 36%formaldehyde sterilised by passing through a bacterial filter were aseptically added. The mixture was kept under /aseptical condition for 72 hr. at18°C. After about 30min. turbidity starts appearing and this increases with time and a thick sediment is formed.

After the said period the mixture was examined ~~examined~~ for sterility by Petri dish technique and found sterilised. The particles were separated from the environmental medium by centrifugation and washed several times with distilled water. The particles were suspended in water to give 12 mg.of the particles per 1 ml. / 0.1 ml. of this sample was added in 20 ml. of distilled water . 0.05 ml.of this sample was used in 3 ml. of the mixture to study the peroxide-like activity. This contained 3×10^{-3} mg.of the particles.

/ 1 ml. of 1.5×10^{-3}M ascorbic acid solution and 2 ml. of 1.5×10^{-2}M hydrogen peroxide solutions were taken in a spectrometer cell and to this 0.05 ml. of the particle suspension were added . The peroxidase- like activity was determined by the oxidation of ascorbic acid with hydrogenperoxide in its presence as is indicated by the decrease in the peak at 264 mu (14)immediately after adding the particle suspension in the reaction mixture and shaking the mix

c* The mineral solution is prepared by dissolving 2 mg. of each of sodium chlori chloride, potassium sulphate,magnesium sulphate, calcium acetate and dipotassium hydrogen phosphate/ in 10 ml. Of distilled water.

mixture. The reaction was followed for 150 seconds during which most of the ascorbic acid of the mixture was oxidised (A). In another experiment a similar mixture was prepared and the spontaneous oxidation of ascorbic acid was with hydrogen peroxide without the particle suspension was studied (B). 0.05 ml. of the particle suspension was heated in a test-tube till it got dried and the the particles charred. 3ml. of the ascorbic acid - hydrogen peroxide mixture as mentioned above were added in the tube, the mixture shaken and examined spectroscopically (C).

As indicated by the spectroscopic estimation the particle suspension has strong peroxidase activity which is destroyed on heating. These particles have boundary wall and internal structure as shown in the micrograph.

The presence of strong peroxidase-like activity in these particles suggests a mechnism of oxidation of organic substances by the oxygen enriched compounds present in the environment and this may provide the energy needed for the various transformations in the earliest selfperpetuating systems.

Abstract:

The particles formed in the mixture of ammonium molybdate, diammonium hydrogen phosphate, biological minerals and formaldehyde have strong peroxidase like activity as indicated by the oxidation of ascorbic acid with hydrogen peroxide in their presence. This peroxidase-like activity is destroyed on heating.

1) Rate of reaction during 0 – 25 sec.

ml of asahi aw	decrease in absorbance
0·0	0·0
0·1	4·0
0·2	8·5
0·4	20·5
0·6	28
0·8	32
0·9	32

2) Rate of Reaction during 25 – 50 sec,

ml of asahi aw.	decrease in absorbance.
0·0	0·0
0·1	2·5
0·2	6·5
0·4	15
0·6	20
0·8	20
0·9	20
1·0	20
1·2	21
1·4	20.

3) Rate of reaction during 50 – 75 sec.

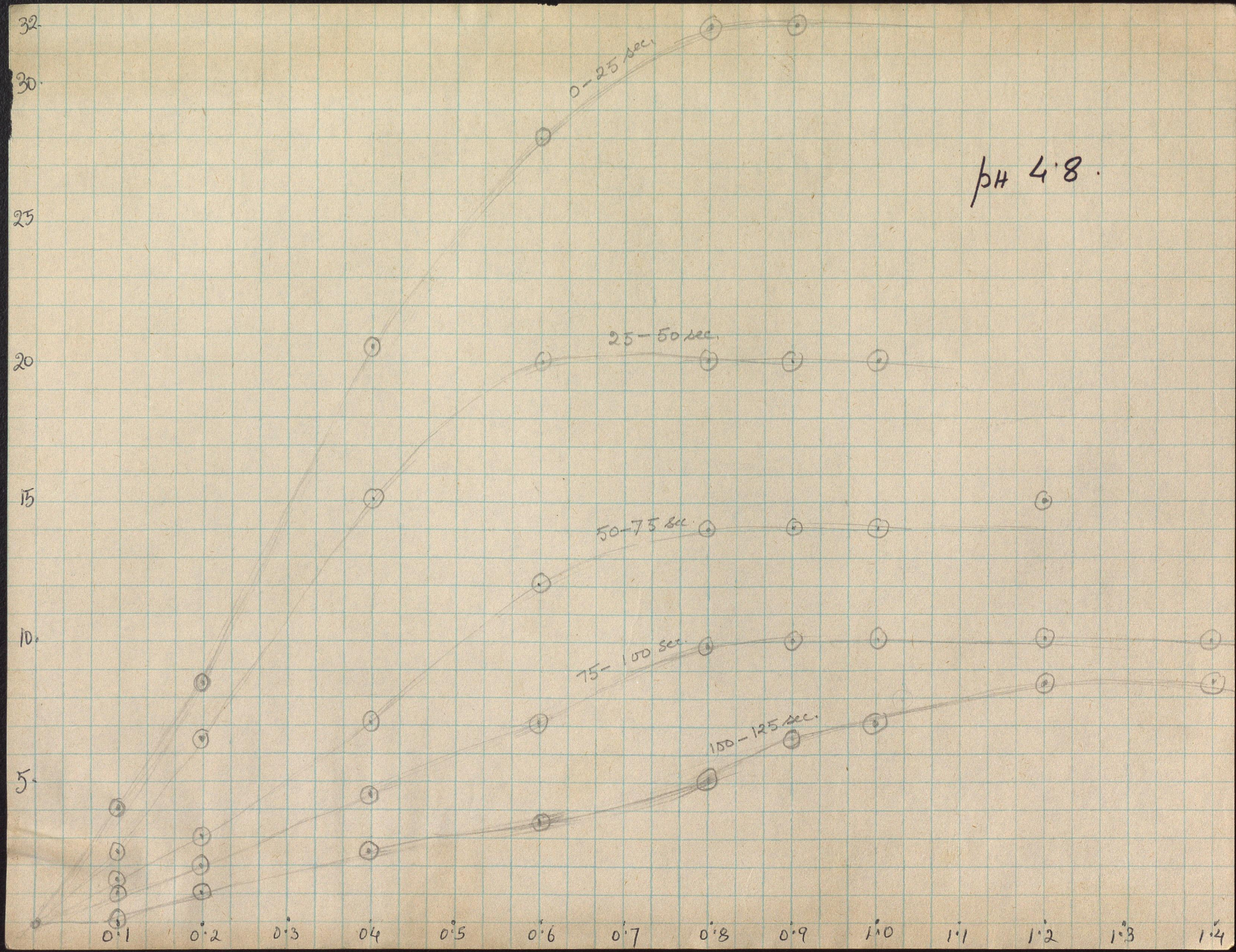
ml. of asahi aw.	decrease in absorbance
0·0	0·0
0·1	1·5
0·2	3
0·4	8
0·6	12
0·8	14
0·9	14
1·0	14
1·2	14
1·4	13
1·6	13

4) Rate of reaction during 75 – 100 sec.

ml. of asahi aw.	decrease in absorbance.
0·0	0·0
0·1	1
0·2	2
0·4	4·5
0·6	7
0·8	10
0·9	10
1·0	10
1·2	10
1·4	10
1·6	11.

5) Rate of reaction during 100 – 125 sec.

ml. of asahi aw.	decrease in absorbance.
0·0	0·0
0·1	0·0
0·2	1
0·4	2·5
0·6	3·5
0·8	5·0
0·9	6·5
1·0	7·0
1·2	8·5
1·4	8·5
1·6	9.


32
30
25
20
15
10
5
0-25 sec.
25-50 sec.
50-75 sec.
75-100 sec.
100-125 sec.
pH 4·8.
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
1·1
1·2
1·3
1·4