

Lawrence Berkeley National Laboratory

Recent Work

Title

ORIGIN OF ENZYMIC AND PHOTOSYNTHETIC ACTIVITY IN A PRE-BIOTIC SYSTEM:

Permalink

<https://escholarship.org/uc/item/6j341252>

Authors

Smith, Adolph E.

Raab, Karl

Mensah, J.A. Ekpaha

Publication Date

1970-10-01

LIBRARY AND
DOCUMENTS SECTION

ORIGIN OF ENZYMIC AND PHOTOSYNTHETIC ACTIVITY IN
A PREBIOTIC SYSTEM

Adolph E. Smith, Karl Raab
and J. A. Ekpaha-Mensah

October 1970

AEC Contract No. W-7405-eng-48

TWO-WEEK LOAN COPY

*This is a Library Circulating Copy
which may be borrowed for two weeks.
For a personal retention copy, call
Tech. Info. Division, Ext. 5545*

LAWRENCE RADIATION LABORATORY
UNIVERSITY of CALIFORNIA BERKELEY

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

**Origin of Enzymic and Photosynthetic Activity in a
Prebiotic System**

Adolph E. Smith,* Karl Raab, and J. A. Ekpaha-Mensan

**Physics Department, Sir George Williams University,
Montreal 107, Canada**

***Present address: Laboratory of Chemical Biodynamics
Lawrence Radiation Laboratory
University of California
Berkeley, California 94720**

The evolution of enzymatic activity from the primitive catalytic activity of small molecules or ions has been the subject of much speculation. Calvin has suggested the decomposition of H_2O_2 as one of the earliest examples of this process (1). Starting as an Fe^{+3} ion catalyzed reaction, it presumably evolved to the catalase catalyzed reaction by successive addition of increasingly complex organic ligands to the iron.

We report that uv irradiation of a solution of NH_4SCN , glycine, and several salts produces insoluble microspheres having peroxidase activity. Glycine has been produced in many prebiotic experiments (1,2), and may be presumed to have been present in a primitive Earth environment. NH_4SCN is a product of juvenile volcanic gases (3). We use artificial sea water (4) as a plausible ionic medium and ferrous ammonium sulfate a convenient source of iron.

In a typical experiment, 40 ml of sea water containing 0.04 moles each of glycine and NH_4SCN and 0.001 moles of $Fe(NH_4)_2(SO_4)_2$ was subjected to uv irradiation at 254 $m\mu$ for three hours from a submerged pen lamp (5). Particles appeared close to the surface after only two or three minutes of irradiation. The reaction was left standing for 14 hours; during this time the particle color changed from beige to grey, suggesting that dark reactions occurred. The particles were separated from the solution on a 0.22 μ filter and were washed repeatedly with distilled water.

Microscopic examination of the particles revealed that they were spherical, 0.2 to 1.0 μ in diameter, and that the average diameter increased with irradiation time. Morphological integrity remained

after heating in boiling water and freezing. Thus stability under geological conditions seems plausible. A scanning electron micrograph is shown in Fig. 1.

Because the microspheres were insoluble in common solvents, we used an aqueous suspension in the peroxidase assay. The suspension was subjected to ultrasonic vibration to obtain a uniform dispersion. We used a modification of a standard peroxidase assay (6). To 10 ml of citric acid-phosphate buffer, 1 ml of 1% H₂O₂, and 1 ml of 1% o-phenylenediamine, we added 1 ml of a 0.2 mg/ml suspension of microspheres. This concentration gave an initial linear increase in absorption at 450 m μ (due to the oxidation of the amine) which allowed convenient determination of relative reaction rates. The pH optimum was 5 (Fig. 2); peroxidase from oats has an optimum of 4.8 to 5.3 (7). The unirradiated solution and the filtrate from the product mixture had strong activity but the final washings from the product had none.

Elemental analysis of the particles revealed C 10.9%, N 14.4%, H 1.22%, S 44.28%, and Fe 18.8%, leaving 10.8% unidentified, presumably oxygen. Since the yield was about 30 mg, essentially all of the iron was incorporated into the particles. An ir spectrum showed strong absorption at 2085 cm⁻¹, indicative of an SCN structure. The ir spectrum and the C, H, N, S atomic ratios of 1.0:1.3:1.1:1.5 suggest a thiocyanate polymer (8) as a major component of the particles. Indeed, the insoluble residue remaining after evaporation of the acid hydrolysate preceding amino acid analysis showed the characteristic brick-red coloration of parathiocyanogen, (SCN)_n.

Although glycine and several other amino acids were identified in the acid hydrolysate, they represent a very small percentage of the total mass of the particles. However, preparations without glycine produced very small yields of particles with a higher iron content than usual, yet with only 2 to 5% of the catalytic activity of the usual particles. Similarly, preparations without iron gave low yields of particles having no detectable peroxidase activity. Catalytically active particles retained their activity after heating in boiling water, which is geologically reasonable. Coincidentally, the peroxidases are among the most stable of all enzymes (9). This data is summarized in Table 1. We could not demonstrate enhanced iron catalytic activity due to its presence in the microspheres.

Iron(III) readily forms complexes with chelating amine ligands (10). The glycine-iron complex in our starting solution appears to be crucial to particle formation as well as to the catalytic effectiveness of the product. However, the analytical data indicate that only a small portion of the iron may be present in this form. Oxides and sulfides would account for the bulk of the iron.

Granick has proposed a model of a primitive photosynthetic unit consisting of oxides and sulfides of iron (11). According to his model, organic compounds could form on the surfaces where hydrogen and hydroxyl ions were utilized. We observed a significant drop in pH, up to 2.5 units, after we irradiated our particle suspension in water for one hour with the uv pen lamp. This change was reversible in several hours in the presence of the particles, but not so if the particles were removed by filtration. Boiling the supernatant did not alter the pH; thus dissolved, H_2S , for example, could not be responsible. If a large amount

of hydroxyl ions were consumed at the surface, then the pH would decrease as in our system.

In order to further explore the similarities between a primitive photosynthetic system and our microspheres, we irradiated the particle suspension and then allowed to stand for several hours until the pH returned to its former value. On further irradiation, the pH decreased once again: a procedure that could be repeated several times. It is interesting to note that a simple solution of ferrous ammonium sulfate will also show a decrease of pH on irradiation, but this effect is not reversible.

The behavior of our particles suggests that we may have found a model similar to the one proposed by Granick. In our microspheres, the iron associates with the organic complexes produced and shows strong catalytic activity as proposed by Calvin (1). In support of this scheme, we found that particles isolated after only thirty minutes of irradiation are very small. Preliminary results indicate that succinic acid, a porphyrin precursor (12) can partially replace glycine in the formation of the microspheres.

Acknowledgments

We thank the National Research Council (Canada) for financial support and Dr. Richard Lemmon for helpful discussions. This work was also supported, in part, by the U. S. Atomic Energy Commission.

References

1. Calvin, M., Chemical Evolution (Oxford Univ. Press, Oxford, 1969), p. 144.
2. Kenyon, D. H., and Steinman, G., Biochemical Predestination (McGraw-Hill, New York, 1969).
3. Gauthier, A., *Compt. rend. Seance Soc. biol.* 132, 932 (1901); 150, 1564 (1910); Herrera, A. L., *Science* 96, 14 (1942).
4. Dawson, R. M., et al., Data for Biochemical Research (Oxford Univ. Press, New York, 1969), p. 508 (ii).
5. Ultraviolet Products, San Gabriel, Calif., Model SC-11.
6. Wallerstein, J. S., Alba, R. T., Hale, M. G., and Levy, H., *Biochim. Biophys. Acta* 1, 327 (1947).
7. Purr, A., *Biochem. Z.* 321, 1 (1950).
8. Cotton, F. A., and Wilkenson, G., Advanced Inorganic Chemistry (Interscience, New York, 1966), p. 314.
9. Saunders, B. C., Holmes-Siedle, A. G., and Stark, B. P., Peroxidase (Butterworths, London, 1964), p. 135.
10. Martin, E. B., Introduction to Biophysical Chemistry (McGraw-Hill, New York, 1964), Chap. 22.
11. Granick, S., in Evolving Genes and Proteins, V. Bryson and H. J. Vogel, Eds. (Academic Press, New York, 1965), p. 67.
12. Shemin, D., Biosynthesis of Porphyrins, Harvey Lectures 50, 258 (1954).

Table 1. Effect of absence of different components from irradiated solution in production of particles.

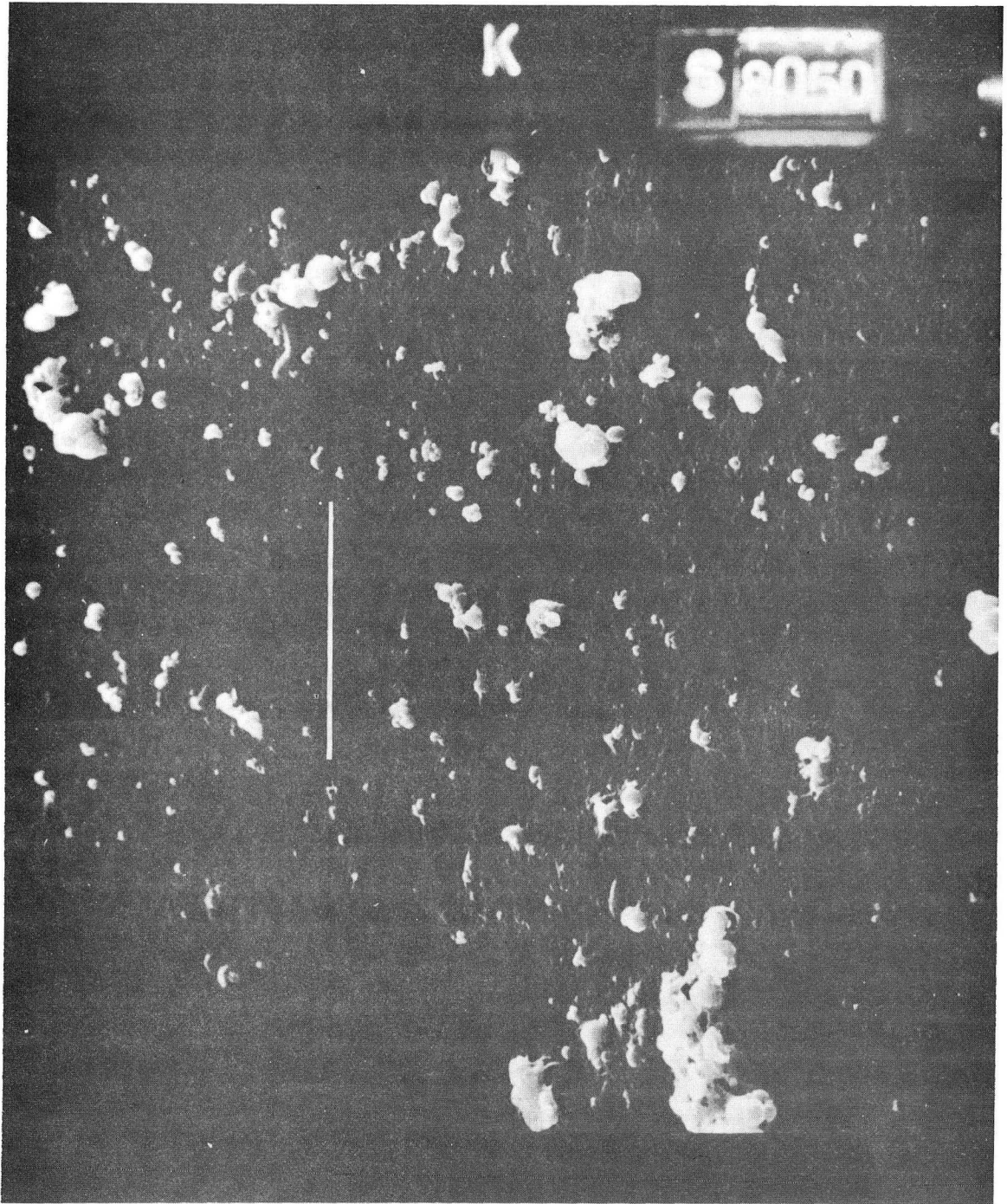
Preparations without sea water salts were run in distilled water. Yield and rate data are based upon the mean values from several experiments.

Component absent in solution	Percent iron in product	Relative yield of product	Relative catalytic action
--	20	1	1
salts	30	0.7	0.5
glycine	30	0.05	0.01
iron	--	0.2	--

FIGURE CAPTIONS

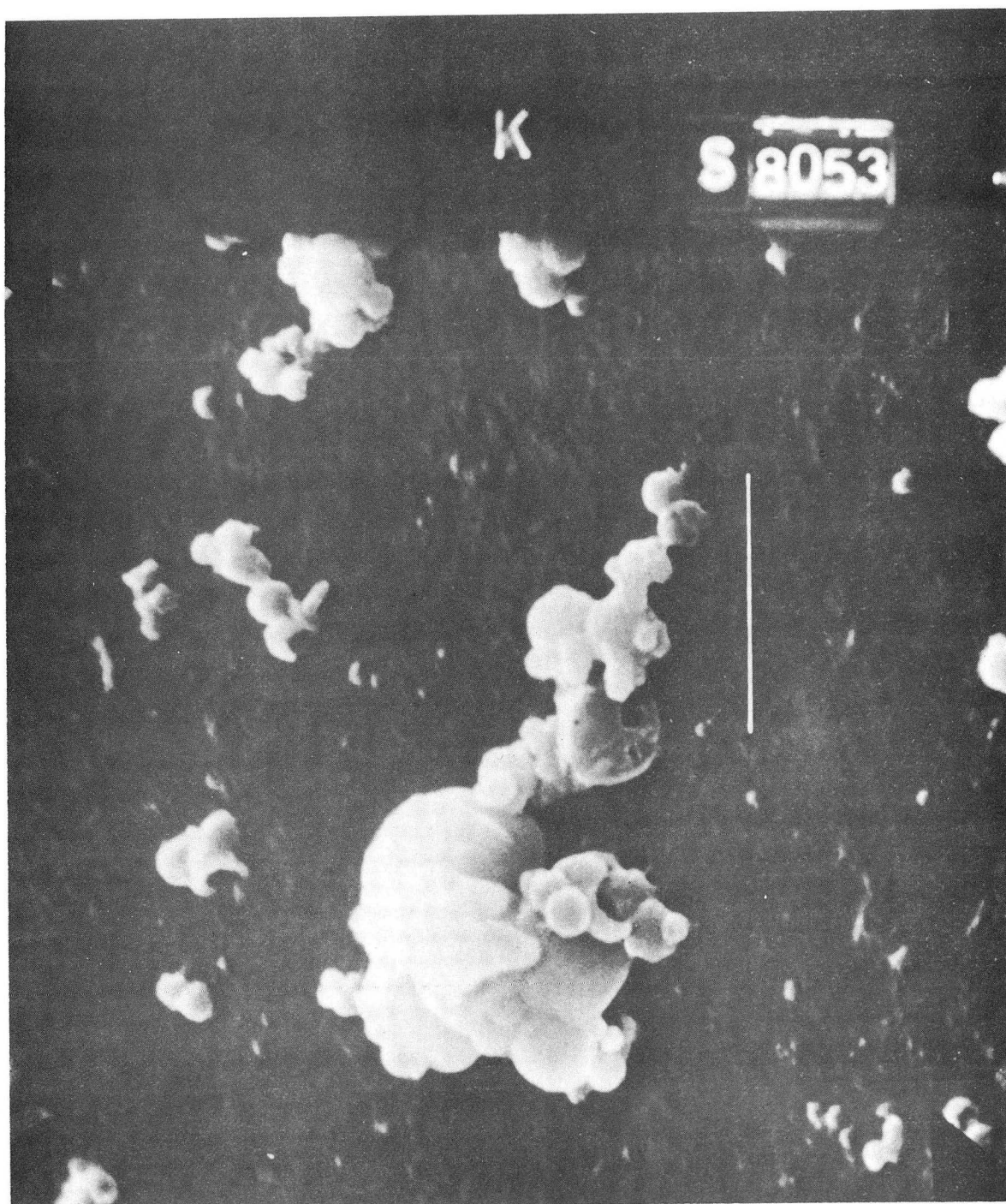
Fig. 1. (a) Scanning electron micrograph of the microspheres. The bar represents 10 μ . (b) Enlarged view. 1 μ is represented.

Fig. 2. pH dependence of the microsphere peroxidase activity.



XBB 7010-4568

Fig. 1(a)



XBB 7010-4569

Fig. 1(b)

1 ml H₂O₂ (1%)

1 ml o-C₆H₄(NH₂)₂·2HCl (1%)

1 ml microspheres (0.2 mg / ml)

10 ml buffer (McIlvaine)

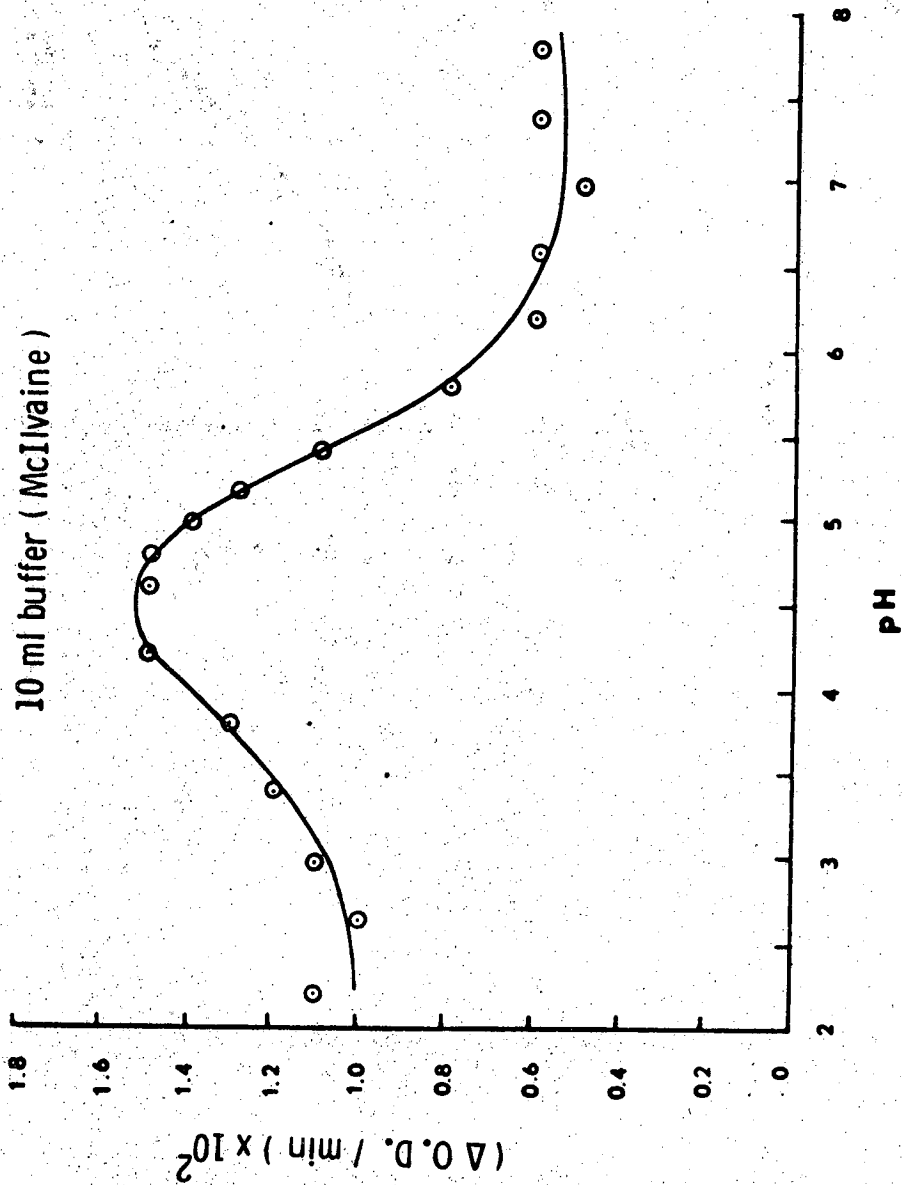


Fig. 2

LEGAL NOTICE

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

- A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or*
- B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.*

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.

TECHNICAL INFORMATION DIVISION
LAWRENCE RADIATION LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720