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ACTIVATION OF GLYCINE BY ATP, A DIVALENT CATION, AND PROTEINOID MICROSPHERES*

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The activation of glycine to yield glycyl hydroxamate has been studied in the absence of enzymes. Activation with ATP in aqueous solution requires only a divalent metal cation. ATP is far more active than other nucleoside triphosphates; AMP and pyrophosphate are inactive. The pH optimum is 4 to 5; activation at pH 7 is most enhanced in the presence of proteinoid microspheres.

A laboratory model for a semipermeable, ultrastructured, replicating protocell composed of partially ordered, catalytically active (proteinoid) macromolecules has been described. Both the polymer and the microsystem have been produced under geologically relevant conditions (Fox, 1969; Fox, 1972; Fox and Dose, 1972). In undergoing replication at the systems level, the proteinoid microsphere grows by accretion (Fox et al., 1967), i.e. by heterotrophic growth (Oparin, 1924; Haldane, 1929; Horowitz, 1945; Van Niel, 1956). A necessary further development for continued evolution toward the contemporary system would have been the replacement of heterotrophic synthesis of peptide bonds by autotrophic synthesis. In the context of this objective, this paper describes the activation of glycine by ATP and a divalent cation. In the primitive environment, proteinoid and proteinoid microspheres would have provided the hypohydrous conditions that have been necessary for such evolution, according to thermodynamic reasoning (Borsook and Huffman, 1944; Dixon and Webb, 1958; Fox, 1968). In the course of the studies recorded, data consistent with the chemical selection of ATP early in evolution have emerged.

The activation of ^{14}C-glycine was demonstrated by conversion of the activated product to ^{14}C-glycyl hydroxamate, which was then separated by high voltage paper electrophoresis and measured on an Actigraph strip counter. The necessity for ATP and divalent cation was shown in a controlled experiment (Table 1), from which components were systematically omitted. As Table 1 indicates, both ATP and divalent cation were essential to the reaction. The result reported with both ATP and divalent cation is similar to that first described by Lowenstein (1958), whose studies were a starting-point for the investigation described here. Lawlor (1967) has however disputed the claim that ATP is essential for the metal ion-catalyzed formation of acethydroxamate. The results in Table 1 of

* This paper is dedicated to the late Aharon Katzir-Katchalsky.

this work are, however, consistent with the findings of Lowenstein, and the latter's interpretation that the reaction is, therefore, a model for biological activation of carboxyl. Indeed, mechanistic considerations suggest that ATP furnishes the necessary energy for the reaction, of which the rate is catalyzed by metal ion. Salts of beryllium, zinc, manganese, magnesium and calcium were also tested in our studies. Beryllium and zinc were the most active, giving in 48 hours yields of approximately 0.1% based on glycine, while magnesium and calcium were least active. The activity of manganese was intermediate. The pH optimum for each metal ion was between 4 and 5.

The questions of whether mono- or diphosphates of adenosine or triphosphates of other nucleosides might serve to activate glycine were examined. ^{14}C-Glycine was again used to yield ^{14}C-glycyl hydroxamate. As revealed in Fig. 1, ATP is by far the strongest activator. AMP yielded no measurable amount of hydroxamate. This result also reaffirms ATP as the source of energy. The absence of strength in pyrophosphate is more surprising, in that ADP, a pyrophosphate derivative, is seen to have some power. Pyrophosphate is known, however, also to be unable to phosphorylate adenosine (Schwartz and Ponnamperuma, 1968). The value for GTP, approximately one-tenth that of ATP, and its superiority to CTP and UTP are of interest because of the involvement of GTP in contemporary pathways (Littlefield and Keller, 1957). Other phosphate derivatives, such as polyphosphates or deoxynucleotide phosphates, were not studied.

Proteinoid microspheres were prepared from acidic proteinoid, basic proteinoid, and calcium (Miquel et al., 1971) in order to provide two kinds of proteinoid. The effect of magnesium ion in association with proteinoid is shown in Fig. 2 at two pH values, 5.0 and 7.0. Magnesium was chosen for this experiment because, of the cations studied, only magnesium does not precipitate ATP at the low concentration of glycine required to minimize dissolution of the microspheres. The larger effects with proteinoid microspheres are found at pH 7.0. The contribution of the magnesium cation is marked. Because there is some dissolution of the microspheres when resuspended in the reaction media, a fully definitive role of the microspheres per se has not been established. However, because of the preponderance of proteinoid in particulate form over that solubilized, the enhancement noted is very likely due to the particulate structures. Whether the reaction occurs in or on the microspheres, and the actual role of each proteinoid component, has not yet been determined. The effect of mixtures of acidic and basic proteinoids in solution has also not been determined, since these mixtures tend to form particles spontaneously.

Table 1
Formation of glycyl hydroxamate by ATP and manganous ion.

Components			CPM of ^{14}C-glycyl hydroxamate
ATP	NH_2OH	$MnCl_2$	
–	+	+	0
+	+	–	0
–	+	–	0
+	+	+	3060

Concentrations were: 0.05 M ATP, 0.5 M glycine containing 100 μc/ml of glycine-1-^{14}C, 0.4 M hydroxylamine, 0.05 M $MnCl_2$. The experiments were incubated at pH 5.0 and 37° for 48 hr.

The geological plausibility of events such as those modelled may be assessed by evaluation of the possibilities for the individual components. Catalytic metal salts, such as included in these studies, certainly occurred on the primitive Earth. The plausibility of the appearance of ATP rests upon the demonstra-

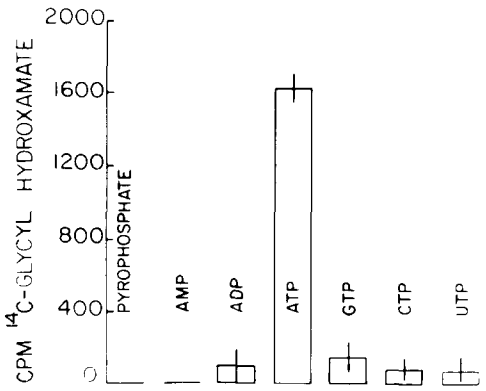

Fig. 1. The formation of glycyl hydroxamate by various phosphate derivatives and $MnCl_2$. Reactants were: 0.5 *M* glycine (including 100 μc/m. of glycine-l-^{14}C), 0.1 *M* hydroxylamine, 0.05 *M* $MnCl_2$, and 0.05 *M* of the phosphate derivatives. Incubation at 37° and pH 5.0 for 24 hours. The averages of duplicate determinations of two experiments are presented.

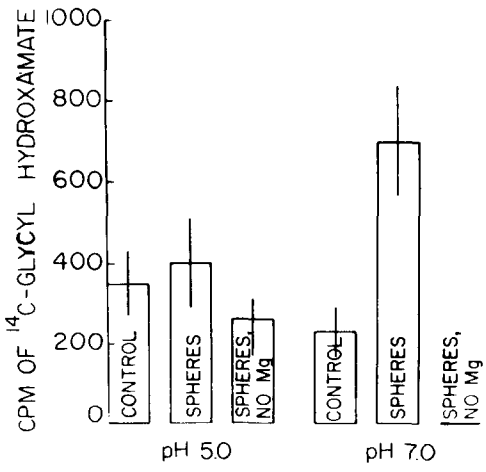

Fig. 2. The effects of acidic proteinoid-basic proteinoid-Ca microspheres on the formation of glycyl hydroxamate. Controls contained: 0.012 *M* glycine (provided by 100 μc/ml of glycine-1-^{14}C), 0.05 *M* hydroxylamine, 0.05 *M* ATP, and 0.05 *M* $MgCl_2$. Other samples also contained the microspheres sedimented from 1 ml of a suspension of acidic proteinoid-basic proteinoid-$CaCl_2$. The samples were incubated at pH 5.0 and 7.0 at 37° for 48 hours. Results are the averages of duplicate assays from three experiments.

tion that adenine (Oro, 1960) and ribose (Pfeil and Ruckert, 1961) could have first arisen. Oro's yield of adenine from HCN was multiplied many times when Wakamatsu et al. (1966) carried out the pentamerization under anhydrous conditions. Fuller et al. (1972) have demonstrated that adenine and ribose are condensable to adenosine in high yield, again when condensed under anhydrous conditions, whereas Fuller et al. observed in water no condensation under a variety of conditions. Adenosine has been phosphorylated to ATP in yields of many percent by Waehneldt and Fox (1967) using polyphosphoric acid, also a product of anhydrous conditions (Harada and Fox, 1965).

Theoretically, the advantages of a cell, or particulate, for activation of amino acids are many. Some of these advantages include (a) the possibility of faster reactions; (b) the possibility of evolution of the proteinoids to powerful enzymes, since enzymelike activities have been demonstrated for these polymers (Oshima, 1971); (c) the general utility for evolution of a peptide-bond synthesis maintained in proximity to supporting reactions by the structure of the cell (Fox and Dose, 1972), and the maintenance of a thermodynamically favorable hypohydrous environment (Fox, 1968).

Although the geological relevance of the specific type of particulate used here has not been fully evaluated, the laboratory experiments (Vegotsky, 1972; Fox and Dose, 1972) indicate that microspheres from acidic proteinoid would have arisen easily and often. The resultant evolutionary flowsheet (Fox and Dose, 1972) is that of amino acids→protoprotein→protocell→(nucleic acid-governed) contemporary cell. The general utility of minimal cells in biochemical evolution has also been pointed out by others (Wald, 1954; Ehrensvard, 1962; Lehninger, 1970). The evolution of an original geochemically produced prototprotein to a cellularly synthesized protein can be visualized, both processes occurring under thermodynamically favorable conditions. Reactions occurring at surfaces (e.g.

Paecht-Horowitz, 1971) are not reactions of dilute aqueous solution.

Inferences about the role of the cell in primordial stages of evolution can be drawn from model constructions, but not so well from reductionistic studies (Mayr, 1964; Grobstein, 1969; Fox, 1973), since the first total entity to be destroyed in analyses of contemporary unicellular life is the organized cell.

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References

Borsook, H. and H.M. Huffman, 1944, Some thermodynamical considerations of amino acids, peptides, and related substances, in: Chemistry of the Amino Acids and Proteins, ed. C.L.A. Schmidt (Charles C Thomas, Springfield, Ill.), pp. 822-870.

Dixon, M. and E.C. Webb, 1958, Enzymes (Academic Press, New York), pp. 666-670.

Ehrensvard, G., 1962, Life: Origin and Development (University of Chicago Press, Chicago).

Fox. S. W., 1968, Self-assembly of the protocell from a self-ordered polymer, J. Sci. Ind. Res. 27, 267

Fox, S.W., 1969, Self-ordered polymers and propagative cell-like systems, Naturwissenschaften 56, 1.

Fox, S.W., 1972, Evolution from amino acids: lunar occurrence of their precursors, Ann. N.Y. Acad. Sci. 194, 71.

Fox, S.W., 1973, Origin of the cell: experiments and premises, Naturwissenschaften 60, 359.

Fox, S.W. and K. Dose, 1972, Molecular Evolution and the Origin of Life (W.H. Freeman and Co., San Francisco).

Fox, S.W., R.J. McCauley, and A. Wood, 1967, A model of primitive heterotrophic proliferation, Comp. Biochem. Physiol. 20, 773.

Fuller, W.D., R.A. Sanchez, and L.E. Orgel, 1972, Studies in prebiotic synthesis VI. Synthesis of purine nucleosides, J. Mol. Biol. 67, 25.

Grobstein, C., 1969, Organizational levels and explanation, J. Hist. Biol. 2, 199.

Haldane, J.B.S., 1929, The origin of life, in The Rationalist Annual, in J.D. Bernal, The Origin of Life (World Publishing Co., Cleveland), pp. 242-249.

Harada, K. and S.W. Fox, 1965, Thermal polycondensation of free amino acids with polyphosphoric acid, in: The Origins of Prebiological Systems and of their Molecular Matrices, ed. S.W. Fox (Academic Press, New York), pp. 289-298.

Horowitz, N.H., 1945, On the evolution of biochemical syntheses, Proc. Natl. Acad. Sci. U.S. 31, 153.

Lawlor, J.M., 1967, Metal ion-catalyzed condensation of acetic acid with hydroxylamine in aqueous solution, Chem. Commun. 1967, 404.

Lehninger, A.L., 1970, Biochemistry (Worth Publishers, Inc., New York).

Littlefield, J.W. and E.B. Keller, 1957, Incorporation of carbon-14-amino acids into ribonucleoprotein particles from the Ehrlich mouse ascites tumor, J. Biol. Chem. 224, 13.

Lowenstein, J.M., 1958, Nonenzymic formation of hydroxamates catalyzed by manganous ion, Biochim. Biophys. Acta 28, 206.

Mayr, E., 1964, From molecules to organic diversity, Federation Proc. 23, 1231.

Miquel, J., S. Brooke, and S.W. Fox, 1971, Assembly of microspheres from acidic proteinoids and histones or histone-like proteinoids, Currents Mod. Biol. 3, 299.

Oparin, A.I., 1924, The Origin of Life (in Russian; Moscow Izd. Moskovskii Rabochii).

Oro, J., 1960, Synthesis of adenine from ammonium cyanide, Biochem. Biophys. Res. Commun. 2, 407.

Oshima, T., 1971, Catalytic activities of synthetic polyamino acids, Viva Origino 1, 35.

Paecht-Horowitz, M., 1971, in: Chemical Evolution and the Origin of Life, ed. R. Buvet and C. Ponnamperuma (North-Holland, Amsterdam), pp. 245-251.

Pfeil, E. and H. Ruckert, 1961, Formaldehyde condensation. Formation of sugars from formaldehyde by the action of alkalies, Ann. Chem. 641, 121.

Schwartz, A. and C. Ponnamperuma, 1968, Phosphorylation on the primitive Earth. Phosphorylation of adenosine with linear polyphosphate salts in aqueous solution, Nature 218, 443.

Van Niel, C.B., 1956, in: The Microbe's Contribution to Biology, A.J. Kluyver and C.B. Van Niel (Columbia University Press, New York).

Vegotsky, A., 1972, in: Molecular Evolution: Prebiological and Biological, eds. D.L. Rohlfing and A.I. Oparin (Plenum Press, New York), pp. 449-458.

Waehneldt, T.V. and S.W. Fox, 1967, Phosphorylation of nucleosides with polyphosphoric acid, Biochim. Biophys. Acta 134, 1.

Wakamatsu, H., Y. Yamada, T. Saito, I. Kumashiro, and T. Takenishi, 1966, Synthesis of adenine by oligomerization of hydrogen cyanide, J. Org. Chem. 31, 2035.

Wald, G., 1954, The origin of life, Sci. American 191 (2), 44.