anodal band of the F_1 hybrid and of the freeze-thaw mixture of parental extracts are identical and that this band represents a hybrid dimer (BB') composed of 1 subunit from each of the most anodal parent dimers. Thus, this in vitro technique apparently produces a molecular hybrid identical to that synthesized in the cells of the interspecific hybrid organism.

Preliminary investigations of our experimental conditions have revealed some similarity between the hybridization of malate dehydrogenase and of lactate dehydrogenase. Hybridization does not occur when salt is omitted from the mixture, when the tissue is homogenized in distilled water, or when 0.1 *M* *Tris*-HCl, pH 8.0, is substituted for the phosphate buffer. There is no apparent difference between quick freezing the mixture and slow freezing by storage at −20 °C for 4 h. This behavior is analogous to that reported for lactate dehydrogenase[15], suggesting a similarity in the mechanism of subunit assembly in these dehydrogenases.

The freezing and thawing process does not greatly reduce the amount of enzyme activity detected by staining. It is possible that an even better recovery of enzyme activity will be obtained by the use of coenzyme as a stabilizing agent as in the freeze-thaw hybridization of lactate dehydrogenase[15]. NADH has markedly enhanced the recovery of malate dehydrogenase during reactivation after acid or guanidine treatment[13]. Like lactate dehydrogenase, variation in the concentration or nature of the hybridization promoting ions may also increase the yield[15]. With optimal conditions, this technique should prove useful in purification procedures and in the preparation of molecular hybrids for the investigation of subunit interactions[16].

Molecular hybridization can be useful in studying protein evolution by examining the affinity of monomeric polypeptides from widely divergent organisms in forming multimeric enzymes[12]. The formation of such hybrid molecules implies a high degree of homology in higher order protein structure. Preliminary results suggest that evolutionary analyses of MDH polypeptides can be performed by the freeze-thaw technique. We have also obtained evidence that the supernatant malate dehydrogenase subunits from the sheep and the largemouth bass assemble into the same dimer during freeze-thaw hybridization.

The present investigation is, to our knowledge, the first demonstration that the freeze-thaw technique produces molecular hybrids of malate dehydrogenase isozymes. This convenient and economical procedure should prove useful in the study of genetics, enzyme structure, and molecular evolution[17].

Résumé. Les hybrides moléculaires des isozymes de la déshydrogénase de malate de différentes espèces de poissons peuvent être engendrés in vitro en gelant puis dégelant les isozymes en présence du sel. L'isozyme hybride est identique à celle observée in vivo dans l'hybride interspécifique F_1.

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Origin of Enzymic and Photosynthetic Activity in a Prebiotic System

The evolution of enzymatic activity from the primitive catalytic activity of small molecules or ions has been the subject of much speculation. CALVIN[1] has suggested the decomposition of H_2O_2 as one of the earliest examples of this process. 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 nm for 3 h from a submerged pen lamp[5]. Particles appeared close to the surface after only 2 or 3 min of irradiation. The reaction was left standing for 14 h; 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 μm filter and were washed repeatedly with distilled water.

Microscopic examination of the particles revealed that they were spherical, 0.2–1.0 μm 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 Figure 1.

Because the microspheres were insoluble in common solvents, we used an aqueous suspension in the peroxidase

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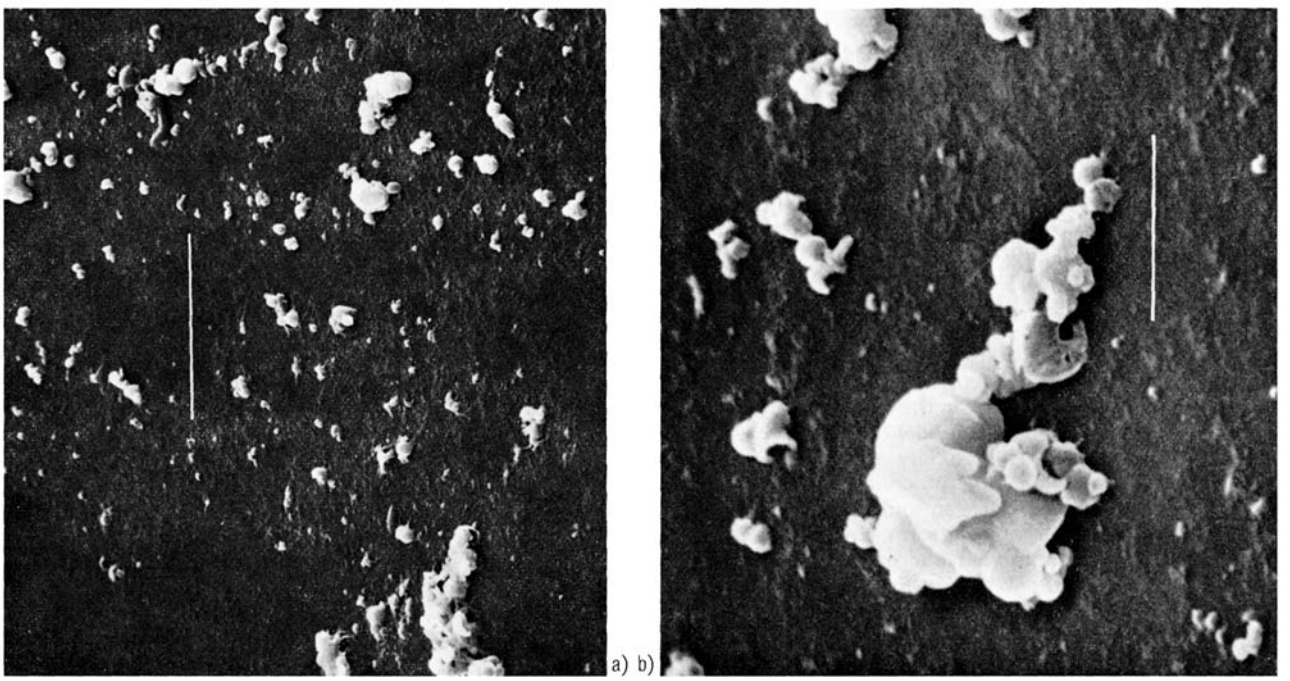

Fig. 1. a) Scanning electron micrograph of the microspheres. The bar represents 10 μm. b) Enlarged view. 1 μm is represented.

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% H_2O_2, and 1 ml of 1% *o*-phenylene-diamine, we added 1 ml of a 0.2 mg/ml suspension of microspheres. This concentration gave an initial linear increase in absorption at 450 nm (due to the oxidation of the amine) which allowed convenient determination of relative reaction rates. The pH optimum was 5 (Figure 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 2–5% of the catalytic activity of the usual

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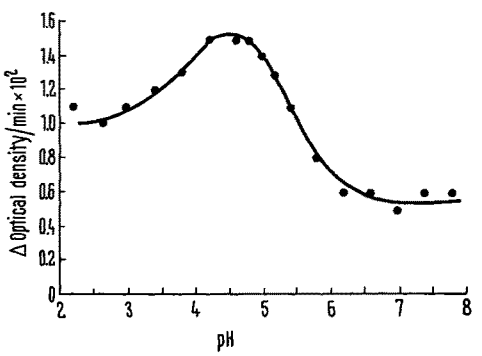

Fig. 2. pH dependence of the microsphere peroxidase activity. 1 ml H_2O_2 (1%); 1 ml *o*-$C_6H_4(NH_2)_2 \cdot 2HCl$ (1%); 1 ml microspheres (0.2 mg/ml); 10 ml buffer (McIlvaine).

Effect of absence of different components from irradiated solution in production of particles

Component absent in solution	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	–
UV	no microspheres produced		

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

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 the Table. 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[11] has proposed a model of a primitive photosynthetic unit consisting of oxides and sulfides of iron. 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 1 h 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[11]. 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 30 min 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[13].

Résumé. L'irradiation UV d'une solution de sulfocyanate d'ammonium, glycine et fer dans l'eau de mer artificielle a produit des microsphères semblables à des cellules douées d'activité catalytique péroxidasique. La glycine a augmenté l'activité catalytique du fer, comme cela purrait s'être produit au cours de l'evolution dans la première étape de cette réaction de catalyse par le fer. On a observé des faits semblables avec un modèle à élément photosynthétique primitif.

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Intracellular Recording of Electrical Activity in Smooth Muscle of the Common Bile Duct[1]

Knowledge concerning the motility of the bile duct is still quite fragmentary and there has been no publication of intracellular measurements of the membrane potential, the most sensitive indicator of the electrical processes combined with excitation of smooth muscle cells. We therefore included the common bile duct of the guinea-pig in a programme of comparative studies on different types of spontaneously active smooth muscle preparations (taenia coli[2], stomach[3], portal vein[4] and ureter[5] of the guinea-pig).

Materials and methods. A length of 10–15 mm of the common bile duct was removed from the junction of the cystic and hepatic ducts up to approximately 2 mm before its passage into the duodenum, and the lumen was immediately cleared from the bile. One end of the preparation was pulled over a perspex cone and fixed with a thread, very similar to the technique used for measurements in the portal vein[4]. The size of the cone was selected in order that the tissue was just sufficiently stretched to prevent movements in this area, and, therefore, long-term intracellular recordings with glass microelectrodes could be performed. The greater part of the preparation was kept under normal tension conditions, stretched to its approximate length in situ. The free end was connected to a transducer for recording the tension development. The preparation was kept in an organ bath at 35 °C, perfused with Krebs solution aerated with 95% O_2 and 5% CO_2. Technical details are given more fully in preceding papers[2,4]. The described partial fixation of the bile duct preparation does not disturb the normal spontaneous activity, as could be shown by experiments where the electrical activity was recorded with extracellular electrodes without any special fixation (will be published separately).

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