Carbon dioxide reduction and nitrogenase activity in organo-molybdenum microstructures[1]

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Summary. Organo-molybdenum microstructures were prepared by visible light irradiation of ammonium molybdate, formaldehyde, ammonium phosphate and a mineral solution. These microstructures are shown to be capable of carbon dioxide photoreduction, nitrogenase activity, and water decomposition, and may represent a metabolic stage between the nonliving and the living.

Functions distinctive of biological photoautotrophs are the decomposition of water, and carbon dioxide and nitrogen reduction. We report here that synthetic cell-sized microstructures of mineral-organic complexes can photocatalyze these reactions and suggest that primordial metabolism could have evolved from a simple rather than a complex chemical environment.

Transition metal photocatalysts have been intensively studied in recent years for their potential as mediators in solar conversion schemes. The photocatalytic reduction of CO_2 under reduced oxygen conditions has been reported[2]. Photocatalytic reduction of dinitrogen mediated by TiO_2 and correlated with water decomposition was demonstrated by Schrauzer and Guth[3]. Bickley and Vishwanathan[4] have recently shown that UV radiation can fix molecular nitrogen at room temperature on the surface of rutile. Dickson[5] used p-GaP electrodes in a photochemical cell to reduce nitrogen under mild ambient conditions, while Hallman[6] used this semiconductor to reduce CO_2 in aqueous solution. Getoff[7] used ferrous sulfate to photoreduce aqueous CO_2 to formaldehyde. It is also well established that dinitrogen can be fixed by reducing metals and metal complexes[8]. In the case of nitrogenase, reduced molybdenum is thought to be involved. A number of systems which simulate nitrogenase and which employ molybdenum with ligands of amino acids or small peptides have been studied by Schrauzer and coworkers[9].

In light of the above considerations, we decided to study the microstructures ('Jeewanu') reported by Bahadur in a long series of articles[10]. Jeewanu are cell-sized (1-3 μm diameter) mineral-organic complexes which form in high yields when solutions of ammonium molybdate, ammonium phosphate, formaldehyde and other minerals are subjected to intense visible light. Hall et al.[4] have also reported that these structures together with a hydrogenase could liberate hydrogen from water when irradiated with visible light. Therefore, it seemed plausible to ask whether these microstructures could photocatalyze CO_2 and N_2 reduction and water dissociation.

Microstructures were prepared in the following manner: 100 ml of a 4% ammonium molybdate solution, 200 ml of a 3% diammonium phosphate solution, 100 ml of a mineral solution and 100 ml of 37% formaldehyde were combined in a 1-l flask. The mineral solution above was prepared by successively dissolving in 100 ml of distilled water 20 mg each of calcium acetate, sodium chloride, potassium dihydrogen phosphate, magnesium sulfate, manganese sulfate and potassium sulfate. 50 mg of ferrous sulfate was added to this mixture. The combined solutions were then exposed to sunlight for 8 h for 3 days, or were exposed to a xenon lamp for equivalent times. After the irradiation period, the turbid dark blue mixture containing microstructures was harvested by filtration using a 0.3 μm filter. Washed structures were dried in a dessicator at room temperature. Typically, 3.5 g of dried particles were obtained and were used in experiments on carbon dioxide reduction, nitrogen reduction and water decomposition. The dried particles had the following percentage composition: carbon 13.75, hydrogen 2.62, oxygen 28.96, nitrogen 8.84, molybdenum 44.50, iron 0.18. IR-spectra of the particles showed strong absorption bands at or close to the principal absorption bands of formaldehyde and ammonium molybdate. These data suggest that the particles are composed of insoluble formaldehyde polymers combined with some form of insoluble molybdate ions. A scanning electron micrograph of the particles is shown in figure 1.

Tests for CO_2 reduction were carried out separately using visible or UV radiation. In the visible radiation experiments, CO_2 was obtained from a Kip generator and bubbled through microstructure suspensions of varying concentrations into 100 ml of aqueous 0.16 N NaOH. The effluent gas from the irradiated suspension was led to a 150-ml distilled water trap at room temperature. 5-ml aliquots were withdrawn from the water trap and titrated with neutral $KMnO_4$ and then acidic $KMnO_4$. A set of nonirradiated controls with bubbling CO_2 was also prepared. The distilled water containing the evolved gases from the irradiated suspension decolorized bromine water, indicating that unsaturated carbon-carbon compounds were present. Results are presented in figure 2. The amount of unsaturated carbon compounds for the non-irradiated controls was very small.

CO_2 reduction was also observed using UV (254 nm) irradiated microstructures. ^{14}C-labeled carbonate was used in these experiments to determine whether reduced carbon was obtained from dissolved carbon dioxide or from the photodegradation of pre-existing organic compounds in the particles. 10 mg of microstructures were suspended in 1 ml distilled water in a septum stoppered 18 × 200 mm quartz tube. The head space was flushed 3 times with N_2 prior to filling with a N_2 (600 mm)-H_2 (50 mm) mixture (one of the simplest presumed primordial atmospheres). Sodium ^{14}C-

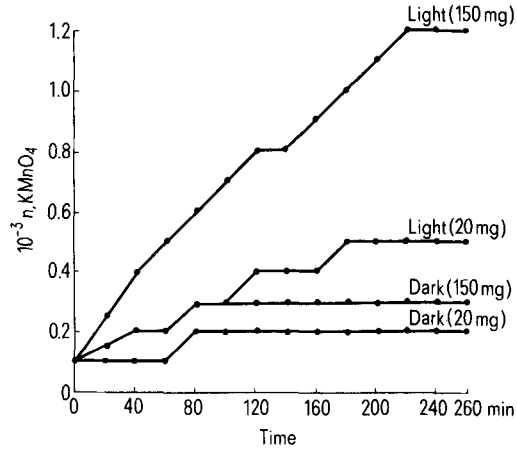

Fig. 1. Scanning electron micrograph of organo-molybdenum microstructures.

carbonate (0.25 ml of $Na^{14}CO_3$ solutions of 50 mCi/mM sp. act., 0.1 ml = 0.075 mCi) was added by syringe. Reaction mixtures were irradiated for 68 h in a Rayonet UPR 2537 photochemical reactor: nonirradiated control mixtures were shielded with several layers of aluminum foil and carried throughout the procedure. After the irradiation period, the suspension was brought to pH 2 with 6 N HCl and the mixtures were outgassed under vacuum and flushed 3 times with N_2. The samples were centrifuged at 15,000 × g for 3 min and the pellet was washed 3 times in distilled water, decanted and counted. Microstructure reactions showed a ^{14}C count 3 times above background, whereas nonirradiated controls possessed no detectable activity. These results indicate that UV radiation (254 nm) will drive apparent carbon fixation from carbonate into the acidified nonvolatile organic fraction. More recent experiments indicate that about 70% of the total ^{14}C activity in the pH 2 acidified sample is sedimentable, while 30% remains as apparent soluble organic compounds. Studies to identify the chemical nature of these ^{14}C-labeled organic compounds are in progress.

Several experiments to test for nitrogenase activity were carried out. Acetylene reduction, driven by either visible or UV radiation, and direct tests for reduction by visible radiation were performed.

For UV (254 nm) driven acetylene reduction, quartz tubes 18 × 160 mm were used. 5 mg microstructures were suspended in 2 ml water or in 2 ml 0.1 M glucose. The head space in the tube was flushed with N_2 and filled with 0.25 ml acetylene at STP. The ratios of acetylene to ethylene were measured by comparing peak heights from gas chromatograms (3 mm × 2 m Porapak T column). The irradiated microstructure suspensions showed significant increases in the ethylene-acetylene ratio (1–3 μM/h/mg ethylene was formed), while the nonirradiated control showed no detectable increase over background.

Acetylene reduction tests were also performed using visible (xenon lamp) radiation of microstructure suspensions. Here too, reduction of acetylene significantly above that of the nonirradiated controls was observed, but the rate of ethylene generation was about $^1/_{10}$ of the UV radiation experiments.

Photoreduction of atmospheric nitrogen by microstructures was also directly tested by bubbling nitrogen through a 500-mg particle preparation suspended in 400 ml of a 5% (v/v) mineral solution prepared as described above. Micro-Kjeldahl tests of the organic nitrogen were carried out daily. Here, the effect of the ambient atmosphere on nitrogen reduction was tested by bubbling a nitrogen stream through one flask and sweeping an air stream above the other. A small effect of oxygen on the reduction rate was observed. Results are shown in figure 3 and demonstrate directly nitrogen fixation.

These results, together with those for acetylene reduction, strongly suggest that microstructures photoreduce dinitrogen. Experiments using $^{15}N_2$ are in progress.

To determine the major source of protons during the photoreduction of acetylene (and by implication other photoreductions reported here), we used D_2O (^2H_2O) instead of H_2O. 10 mg of microstructures were placed in 1 ml of 99% D_2O in 16 × 160 mm quartz tubes stoppered with septum caps. Tubes were evacuated and flushed 3 times with N_2: acetylene to 200 mm STP was added. Tubes were irradiated in the photochemical reactor for 24 h. Ethylene was separated from acetylene by gas chromatography (Porapak T 3 mm × 2 m column) and enriched for online mass spectral analysis by a 2-stage membrane separator at room temperature. Mass spectrometry was conducted using 20 ev EI source, resolution of 2800 (10% valley), source temperature of 150 °C, and scan times of 2 sec per m/e range of 12–60. In all cases, the sensitivity of the mass spectrometer was set so the ethylene fragment ions of interest were near 50% of full scale deflection on the 100X galvanometer at the ethylene GC peak maximum. At least 5 spectra were recorded for all samples, data were normalized, checked for skew, and averaged before calculation for D enrichment. D atom % excess was calculated for ethylene fragment ion pairs m/e 13 and 14 and found to be 43% above that for a standard ethylene. Hence at least 86% of the ethylene product molecules bore the deuterium label. Although it is difficult to obtain quantitative results under these conditions (the deuterium label can itself redirect parent ion fragmentation patterns), it is safe to conclude that the majority of the acetylene which was reduced to ethylene had obtained its protons form D_2O.

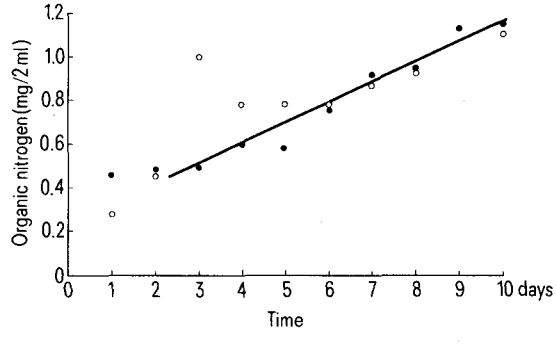

Fig. 2. Tests for CO_2 reduction by the titration of distilled water trap (5 ml aliquots) containing evolved gases from CO_2 bubbled-visible light exposed mineral organic microstructure suspensions against 10^{-3} N $KMnO_4$.

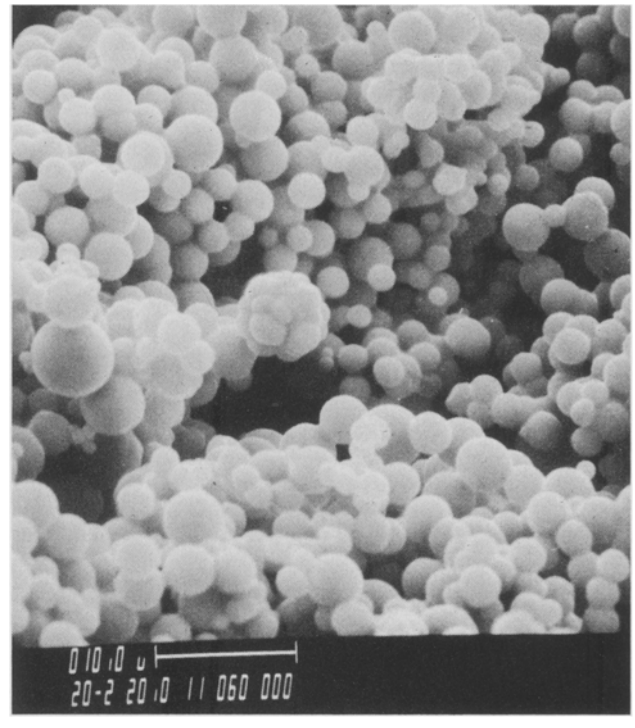

Fig. 3. Photoreduction of nitrogen by mineral-organic microstructures. Micro-Kjeldahl organic nitrogen as mg/2 ml reaction mixture. See text for methods. ●, under nitrogen; ○, under air. All values averages of 4 samples.

These conclusions are consistent with the observation of Schrauzer[3].

Our experimental results show that microstructures composed of molybdenum, iron and organic compounds can photocatalyze CO_2 reduction, N_2 reduction and water decomposition. In view of the abundance of reports in recent years which demonstrate that many transition metal complexes alone can perform these photocatalyses[12,13] and from the recent work of Yamase and Ikawa[14], it is not surprising that such catalysis is observed for these microstructures which use several transition metals as starting materials. However, this appears to be the first report for a photocatalytic role of molybdenum in carbon reduction[15].

The important concept which these observations lead to is *not* that these photocatalytic functions can be performed, but rather that these functions can take place concordantly in microstructures formed under geologically plausible conditions. These functions are characteristic of the procaryotes, in particular the blue-green bacteria, and our point is that mineral-organic microstructures can simulate these functions. Therefore, these microstructures clearly represent a likely metabolic stage between the nonliving and the living from the point of view of functional attributes. Hartman's[17] and Folsome's[18] conclusions that metabolism could have evolved from a simple rather than a complex environment is consonant with our findings.

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Niche width of parasites in species-rich and species-poor communities[1]

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Summary. Niche width of ectoparasites of marine fishes, as measured by host range and microhabitat width, is not affected by the number of species in a community. There is no reduction in species numbers of Monogenea due to greater numbers of other parasite species, and frequencies of infection with Monogenea are greater in tropical, species-rich communities.

Latitudinal gradients in species diversity are known for most groups of animals and plants[2]. Opinions differ about the mechanisms which allow so many tropical species to coexist. For theoretical reasons, it is usually assumed that in habitats with many species either the niches are narrower or they are more densely packed[3]. Recently, it has been suggested that a high diversity is maintained by frequent disturbances of intermediate severity[4], or by prevention of competitive equilibrium due to environmental fluctuations leading to periodic population reductions[5]. 'Solution of the problem is difficult because the niche is a highly abstract construct and to measure it is manifestly impossible'[6]. 'We can lower our sights, however, and, for suitably chosen organisms, make observations yielding quantitative measurements that can reasonably be interpreted as measures of niche width and overlap'[6].

In the following, ectoparasites of marine fishes and particularly Monogenea will be used as a model to study the mechanisms which permit coexistence of many species. Such parasites are an almost ideal model for several reasons. 1. The marine environment is more uniform than freshwater and terrestrial environments and gradients superimposed on latitudinal gradients are less important; 2. environmental variability can be further reduced by considering small habitats in the open water, i.e. the surface of fish; 3. the surface of fish and particularly the gills are habitats which can be examined accurately and quantitatively in a short time; 4. the distribution of ectoparasites in certain microhabitats can be accurately mapped; 5. many marine fishes are easily available in large numbers.

Evaluation of data from many surveys showed that there is an increase in species numbers of coastal marine fishes from high to low latitudes, and that species numbers are greater in the Pacific than in the Atlantic Ocean[2,7]. Species numbers of gill Monogenea increase even more strongly towards the equator, and they also are greater in the Pacific than the Atlantic[8]. Hence, communities of ectoparasites on tropical fish and particularly in the Pacific are generally richer in species than communities on cold-temperate fish.

In order to compare niche width of species in communities consisting of many and of few species of ectoparasites, 2 niche components are considered, 1. host range, and 2. size of microhabitat.

ad 1. Rohde[9,10] has shown that Monogenea have a similarly narrow host range at all latitudes, and there may even be a slight widening of the host range in warm Pacific waters,