obtain sufficiently good statistics for the coherent scattering cross sections very large numbers of neutrons have to be counted. We have used a dedicated diffractometer at the High Flux Isotope Reactor to obtain neutron data of very high statistical accuracy for four mixtures of light and heavy water containing 0.01, 35.79, 67.8, and 99.75 percent deuterium (*4*). Another problem is the complex nature of the interaction of thermal neutrons with light nuclei such as hydrogen. Inelastic and recoil effects make extraction of the static coherent scattering function from the measured effective cross sections difficult. We have solved this problem by constructing the dynamic scattering function for sufficiently realistic models of water and integrating over the energy transfers (*5*). The static coherent scattering functions for the four mixtures were then analyzed to yield the three partial structure functions. Subtraction of the intramolecular terms yielded the three intermolecular structure functions for the atom pair interactions in liquid water. The accuracy of these results is very difficult to assess, because they were derived from experimental data with a very low signal-to-noise ratio. However, we have shown (*4*) that the partial structure functions meet all known consistency tests for diffraction data from liquids. The determination of the three atom pair distribution functions by Fourier inversion is complicated by sizable termination effects, which have been minimized by an empirical procedure (*6*). Only a brief description of the main results, shown in Fig. 1, will be given here.

The oxygen atom pair distribution function, $g_{OO}(r)$, derived from the neutron data is not significantly different from that obtained previously from x-ray diffraction (*1*). This curve contains information about the positional correlation between centers of molecules in liquid water. Principal features are an asymmetric distribution of nearest neighbors centered at 2.85 Å, which overlaps with the distribution of tetrahedrally coordinated second neighbors near 4.5 Å. This overlap of first and second neighbor distance distributions has been interpreted (*7*) in terms of a complex near neighbor coordination sphere which cannot be explained by tetrahedral coordination alone.

The function $g_{HH}(r)$ describes the distance distribution between hydrogen atom pairs in different water molecules and, hence, contains information about orientational correlations in the liquid. Principal features are a near neighbor distribution centered at 2.29 Å which

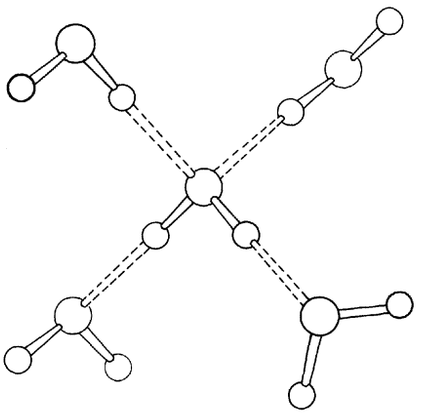

Fig. 2. Model for the average orientation of pairs of near neighbor molecules in liquid water. Large local and instantaneous deviations from this average configuration occur in the liquid.

overlaps with the distribution of second neighbors near 3.9 Å. The function $g_{OH}(r)$ shows a narrow distribution of first neighbors near 3.2 Å. A nearest neighbor O···H distance of 1.86 Å, a corresponding O···O distance of 2.85 Å, and an intramolecular O–H distance of 0.96 Å (*4*) suggest that most water molecules are connected to their nearest neighbors through nearly straight hydrogen bonds as shown in Fig. 2.

The oxygen atom pair distribution function $g_{OO}(r)$ derived from x-ray diffraction has already provided a crucial test of water models in computer experiments (*8*). The functions $g_{OH}(r)$ and $g_{HH}(r)$ derived in this study will provide an even more sensitive test of proposed models for liquid water. This should help lead eventually to a realistic statistical mechanical theory of liquid water.

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Laboratory Cultivation of *Prochloron*, a Tryptophan Auxotroph

Abstract. *Laboratory cultures have been established of the didemnid symbiont* Prochloron, *a unique prokaryotic alga that synthesizes chlorophylls a and b but no phycobilin pigments. Cell division in* Prochloron *cultures occurs under acidic conditions (*p*H 5.5) in the presence of tryptophan. The alga is a naturally occurring tryptophan auxotroph that survives in nature by close association with the host,* Diplosoma similis. *The metabolic dysfunction that renders* Prochloron *auxotrophic may involve only the initial step of the tryptophan biosynthetic pathway.*

The unicellar prokaryotic alga *Prochloron* sp. (*1*), found in nature in symbiotic association with one family of colonial didemnid ascidians (Urochordata), has received considerable attention from botanists. This unusual alga synthesizes chlorophylls a and b and lacks phycobilins, thus resembling higher plants rather than blue-green algae (*2*). The alga has been placed in a new division, the Prochlorophyta, to reflect these characteristics (*3*). Efforts to study this alga have been hampered by the inability of investigators to maintain cultures of *Prochloron*. We report here on conditions that allow the cultivation of *Prochloron*, and offer an explanation for the difficulties encountered in previous attempts at culturing the organism.

Our efforts to establish suitable culture conditions for *Prochloron* were based on attempts to duplicate conditions found in its Hawaiian host, *Diplosoma similis* Fluiter (formerly *D. virens*) (*4*). Since crushed colonies of this didemnid ascidian liberate acid (*5, 6*), it is possible that the algal cells normally exist in an acidic environment rather than the alkaline surroundings in which cyanophytes are commonly observed (*7, 8*). It also appears that *Prochloron* depends on some generally available metabolite rather than a compound of restricted distribution, as the alga has been observed with

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a variety of host didemnid species, growing extracellularly both in the cloacal cavity and on the surface.

Diplosoma similis was collected near Coconut Island, Kaneohe Bay, Oahu. *Prochloron* was freed from the host by mechanically expressing the cells into media buffered with 10 m*M* 2-(*N*-morpholino)ethanesulfonic acid (MES) at *p*H 5.5 (*9*). The volume of inoculum added to test cultures was in no case greater than 100 μl.

The basal medium was a modification of the MN medium of Rippka *et al.* (*10*). Addition of various organic nitrogen and carbon sources (including yeast extract, urea, glucose, citrate, and amino acids) to the basal medium suggested that some amino acid might be required for the growth of *Prochloron*. Each of the 21 "common" levorotatory amino acids was tested as a supplement to the basal medium at a final concentration of 1 m*M*. Of the amino acids tested, only L-tryptophan affected *Prochloron* growth. As shown in Fig. 1, there was a direct relation between tryptophan concentration and division of *Prochloron* cells. Growth in media adjusted to initial *p*H values from 4.5 to 8.0 was also examined. Maximum growth was observed at *p*H 5.5.

The specificity of the amino acid requirement suggested that tryptophan was not serving as a catabolic substrate, since other related amino acids did not stimulate growth. Furthermore, the addition of 1 m*M* L-kynurenine (an early intermediate of tryptophan catabolism) to the basic medium did not support growth of the alga.

Biosynthetically, tryptophan is derived from intermediates of the shikimate pathway, as are tyrosine and phenylalanine. While some differences in the regulation of chorismate mutase (E.C. 5.4.99.5) have been noted between blue-green and green algae (*11, 12*), tryptophan synthesis in *Anacystis nidulans* apparently proceeds as in green algae and higher plants (*12*). Although algal mutants requiring amino acids have been described, *Prochloron* appears to be the first naturally occurring amino acid auxotroph to be reported among the prokaryotic algae.

Since the addition of tyrosine or phenylalanine did not support the growth of *Prochloron*, it appears that some biosynthetic step subsequent to chorismic acid (the final intermediate common to both tyrosine and tryptophan synthesis) and unique to the tryptophan pathway, is impaired.

To test this hypothesis, growth of *Prochloron* was examined in media supplemented with various intermediate compounds of the shikimic acid–tryptophan pathway (Table 1). As predicted, shikimic acid alone did not support growth of the alga. However, since cell membrane impermeability prevents uptake of shikimic acid in *Claviceps* (*13*), we cannot rule out the possibility of a similar situation in *Prochloron*.

Table 1. Growth of *Prochloron* in media supplemented with various metabolic intermediates. Culture conditions and the cell counting method are described in the legend to Fig. 1. Values are means ± standard errors.

Supplement	Cell density (cells per milliliter)
Control (no additions)	1,780 ± 323
Shikimic acid (1 m*M*)	2,100 ± 593
Anthranilic acid (1 m*M*)	20,000 ± 2,478
Indole + serine (1 m*M* each)	21,550 ± 936
Tryptophan (1 m*M*)	20,890 ± 309

Anthranilic acid did support growth, indicating that the metabolic dysfunction that renders *Prochloron* auxotrophic involves the first specific step of tryptophan biosynthesis. In all microorganisms studied to date, this step is catalyzed by anthranilate synthase (E.C. 4.1.3.27), which forms anthranilic acid from chorismic acid and glutamine, with a concomitant release of pyruvic acid. A variety of microorganisms with defective anthranilate synthase have been described, including strains of *Bacillus subtilis* (*14*) and *Neurospora crassa* (*15*).

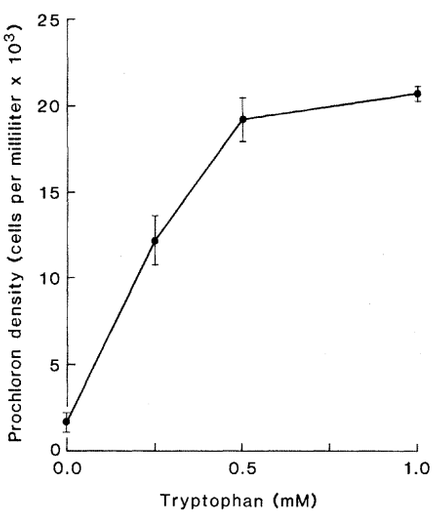

Fig. 1. Relation between tryptophan concentration and *Prochloron* cell division. Each point is the mean ± standard error for five replicate cultures. Cell counts were made with a hemacytometer on duplicate samples taken after thorough mixing. All cultures were incubated for 14 days. Initial cell density was 2000 cells per milliliter in 10 ml of medium. Incubation temperature was 25° ± 1°C and light intensity was 110 μE/m^2 per second from Cool White fluorescent tubes with a regime of 18 hours of illumination and 6 hours of darkness.

Prochloron cells were also able to divide in the presence of indole plus serine (Table 1), indicating that the tryptophan synthase of this alga, like the enzymes of *Neurospora* (*16*) and other microorganisms (*17, 18*), is capable of synthesizing tryptophan from these substrates.

In conclusion, host-free cell division of *Prochloron* sp. occurs at *p*H 5.5 in a seawater medium supplemented with tryptophan, anthranilic acid, or a combination of indole and serine. The growth rates of the alga are slow compared with those of many other algal cultures. However, the growth rate of *Prochloron* in *D. similis* is not known. Experiments to optimize culture conditions for axenic cultures of *Prochloron* would allow a thorough investigation of the physiological and biochemical features of this unusual organism.

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