EFFECT OF HIGH ATMOSPHERIC CO_2 CONCENTRATION ON $\delta^{13}C$ OF ALGAE

A possible cause for the average depletion of ^{13}C in Precambrian reduced carbon.

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Abstract. Precambrian reduced carbon is more depleted in ^{13}C than what would be expected from the carbon isotopic composition of modern marine algae and algal mats. Since the photosynthetic carbon fixation by algae is the most likely source of the reduced carbon, the depletion has been considered an anomaly.

We examined factors that might have contributed to the carbon isotope fractionation from inorganic sources through algae to organic matter in a sedimentary rock, and related laboratory obtainable data to those from Precambrian rocks. Laboratory culture experiments were then performed with nine strains of algae at various concentrations of carbon dioxide, and the result was interpreted according to the relationship.

It indicated that the depletion could be understood in terms of a combined effect of fractionation factors, most depletion occurring at the fractionation during the photosynthetic carbon fixation. It also suggested that all but one algal strain incorporated bicarbonate as the source of carbon for its growth. The exception was a thermophilic, acidophilic alga, which must have used carbon dioxide as the carbon source.

The present study suggests that Precambrian atmosphere was enriched in carbon dioxide roughly two orders of magnitude more than its present atmospheric level.

1. Introduction

An impressive volume of chemical and biological studies on Precambrian rocks has been carried out within the last two decades. The idea that life existed on the Earth as early as the early Precambrian appears to have been established (Nisbet, 1980). The carbon isotopic composition has been one of the criteria used for the determination of the biogenesity of carbon-containing compounds in Precambrian rocks. Since the photosynthetic fixation of carbon preferentially takes up the lighter isotope (Park and Epstein, 1960), the generally low $\delta^{13}C$* values of Precambrian reduced carbon

$$* \ \delta^{13}C\ (‰) = \frac{\left(\frac{^{13}C}{^{12}C}\right)_{\text{sample}} - \left(\frac{^{13}C}{^{12}C}\right)_{\text{standard}}}{\left(\frac{^{13}C}{^{12}C}\right)_{\text{standard}}} \times 1000$$

All carbon isotopic composition given in this paper are presented as a numerical permil difference relative to Peedee belemnite standard (PDB). When an expression $\delta^{13}C_{A/B}$ is used, it is the $\delta^{13}C$ of sample *A* that is obtained from *B*. *B* is not always explicitly given if the source of the sample is obvious.

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were interpreted as to indicate their biological origin.

Upon a close examination, however, one problem concerning $\delta^{13}C$ values remains to be explained if the reduced carbon is really from biological activities in the Precambrian period. In the absence of land plants in Precambrian times, the biological source of the Precambrian reduced carbon is most likely to be the photosynthetic fixation by aquatic algae. Precambrian reduced carbon is, however, isotopically more depleted in ^{13}C as compared with modern aquatic algae.

The $\delta^{13}C_{org/rock}$'s of Precambrian reduced carbon studied mostly cluster between −19‰ and −31‰ (Deines, 1980), whereas the $\delta^{13}C_{org/alga}$ of present aquatic algal organic carbon averages from −18‰ to −20‰. This difference of approximately 6‰ may appear insignificant, but it is not. A similar extent of the difference observed between $\delta^{13}C$ of modern C_3 land plants and that of modern marine algae is solid enough to be used to evaluate the contribution of land-derived organic matters to organic carbon in marine sediments (Hedges and Parker, 1976). Thus, the ^{13}C depletion in Precambrian reduced carbon can be regarded as an anomaly.

Although the anomaly has not yet been explained in a consistent fashion (Towe, 1982), various factors have been considered as a possible cause for it. Among them was a higher CO_2 partial pressure in Precambrian environment. The dependence of carbon isotope fractionation on CO_2 partial pressure of three algal cultures was examined in the laboratory by Calder and Parker (1973). Though they found an increased depletion of ^{13}C at higher CO_2 partial pressure, they thought the depletion insufficient and concluded that blue-green algae might not be a major contributor to the organic matter in Precambrian sediments.

We here present a re-examination of the effect of high CO_2 partial pressure on the algal photosynthetic fractionation of carbon isotopes (Mizutani and Wada, 1982a and 1982b), and like to suggest that a higher CO_2 partial pressure than the present atmospheric level is one of major elements responsible for the depletion of ^{13}C in Precambrian organic carbon.

2. Experimental Methods

2.1 Algal Cultures

Stock cultures of *Anacystis nidulans* (IAM M–6) and *Chlorella pyrenoidosa* (IAM C–101) were obtained from the Institute of Applied Microbiology, University of Tokyo. *Synechococcus* sp. was donated by Professor Sakae Kato of University of Tokyo. *Cyanidium caldarium* strain RK–1, an acidophilic alga, was presented by Professor Ikujiro Fukuda of Science University of Tokyo. Four of five other strains of algae used in this experiment were collected and isolated by Dr Takashi Yamada of Mitsubishi-Kasei Institute of Life Sciences from four different hot springs in Japan. Three of them were thermophilic blue-green algae and one was thermophilic *Chlorella* sp. with the ability of N_2 fixation (Yamada and Sakaguchi, 1980). The last of the five was collected by Dr Masao Minagawa of our laboratory and isolated by us.

TABLE I

Algae and growth conditions

Code	Description	Medium	Temp. (°C)	Light intensity (lux)
A.n.	*Anacystis nidulans*	HMBM*	33	2700
Syn	*Synechococcus* sp.	M. for Syn	57	2700
Cya	*Cyanidium caldarium*	M. for Cya	48	2700
C101	*Chlorella pyrenoidosa*	MBM	35	2700
SAR	filamentous blue-green alga with heterocysts from Sarugakyo Hot Spring	MDM	45	2700
YT	filamentous blue-green alga without heterocysts from Yunodaira Hot Spring	MDM	45	2700
MY	filamentous blue-green alga without heterocysts from Miyanoshita Hot Spring	MDM	45	1000
YUG	unicellular blue-green alga from Yugawara Hot Spring	MDM	45	1000
T2	*Chlorella* sp. from Takaragawa Hot Spring	MDM	45	2700

* The medium was MBM and the temperature 35°C, when A.n. was cultured at one PAL CO_2.

None of the media used contained carbon compounds such as bactopeptone in a substantial quantity that can serve as a carbon source for algae.
MBM: modified Bristol medium, HMBM: highly modified Bristol medium, MDM: modified Detmer medium.

It was a thermophilic unicellular blue-green alga. Table I summarizes algal strains used in this experiment.

2.2 MEDIA FOR ALGAE

Chlorella pyrenoidosa was cultured in a modified Bristol medium (Watanabe, 1960). Five thermophilic algae from Japanese hot springs are all cultured in a modified Detmer medium (Watanabe, 1960). *Anacystis nidulans* was cultured in a highly modified Bristol medium that differs from the modified Bristol medium in the following components (g/l): K_2HPO_4, 0.60; KH_2PO_4, 0.30. In addition, the pH of the highly modified Bristol medium was adjusted to 7.2 with KOH before autoclaving. The medium for *Synechococcus* sp. was that given elsewhere (Hirano *et al.*, 1980). *Cyanidium caldarium* was cultured in a medium described by Enami and Fukuda (1975).

2.3 GROWTH CONDITIONS

Table I shows the growth conditions used for each alga. Carbon dioxide-free air and CO_2 from a cylinder were mixed in a large container and then constantly bubbled through a flat culture flask (volume: 700 ml) at about 500 ml/min. The concentration of CO_2 in the feed gas was measured gas chromatographically and routinely monitored by flow meters. Growth of algae was measured with haemacytometers.

2.4 SAMPLE PREPARATION

Cells were harvested by filtration on a glass filter (Whatman GF/C) preheated at 420°C for at least five hours. They were vacuum desiccated and then decarbonated by an addition of 6N HCl. The filter with algal cells was left undisturbed for two days and later vacuum dried and kept in a desiccator until use. At times the effect of the decarbonation on $\delta^{13}C$ of the algae was examined, and no change was found.

2.5 ANALYSIS

Carbon isotope measurements were made with a dual-collecting Hitachi RMU–6R mass spectrometer, and the isotopic data are reported in units of $\delta^{13}C$ relative to PDB standard. There were two working standards in the present work. Both were 0.5 M sodium bicarbonate solutions whose carbon isotopic compositions were calibrated against National Bureau of Standards isotope reference material No. 20 and No. 21. The $\delta^{13}C$ of one of the two working standards was –19.4‰. That of the other standard was 7.4‰ more positive.

All isotopic composition data presented in this paper were corrected for ^{17}O. Results from a triple-collecting Finnigan MAT–250 of the National Institute of Agricultural Sciences were compared with those from the Hitachi RMU–6R to modify the correction equation of Craig (1957).

A Shinku-Riko Infrared Gold Image Furnace RHL–E210N was used to oxidize the sample. In this procedure, the sample together with the glass filter is placed in a quartz tube with CuO powder that contains a small amount of WO_3 and V_2O_5. The tube was heated at 750°C for six minutes. The gas thus generated passed through two CuO columns heated at 750°C and at 700°C, and through one Cu column heated at 400°C. These columns contained silver granules and/or platinum wires as well as either CuO or Cu. After trapping water with a mixture of dry ice and ethanol, sample CO_2 was condensed in a series of three traps with liquid N_2. The vacuum line is connected to high vacuum three minutes after the heating was terminated and any residual gases not trapped by liquid N_2 were pumped from the line. The line was then isolated from the furnace. Liquid N_2 was replaced by the mixture of dry ice and ethanol, and the CO_2 is transferred into a sample container for mass analysis.

3. Relation between Data from Culture Experiments and Those from Precambrian Rocks

Eichmann and Schidlowski (1975) measured the carbon isotopic compositions of coexisting organic carbon-carbonate pairs covering the whole Precambrian period, and reported the mean difference in $\delta^{13}C$ between two carbon-containing materials: $\delta^{13}C_{org/rock} - \delta^{13}C_{carb/rock} = -25.6‰$. The difference seems anomalously large, since the mean difference between modern aquatic algae and present marine carbonate is about −19‰. In the following argument, we will derive an equation which relates exerimentally obtainable $\delta^{13}C$'s to those from Precambrian rocks and which should be satisfied if the experimental conditions are in some way to reflect the environments in the Precambrian period.

Table II lists factors that may affect the carbon isotopic composition in sedimentary rocks. The nutritional status may affect the composition of higher plants (Bender and Berge, 1979; Boag and Brownell, 1979); however, it is not listed in Table II since the reported fractionation is either small or null and no such effect is so far known for aquatic algae (Pardue *et al.*, 1976). There are other factors of course that are known to alter $\delta^{13}C$ of organic material: notably, the methane bacterial action (Schoell and Wellmer, 1981) and the bacterial dark fixation of carbon (Nakamura, 1982). However, it seems unwarranted to assume that these factors are as ubiquitous as those listed in Table II. Since the depletion of ^{13}C in more than several thousands of Precambrian reduced carbon from a variety of locations (Schidlowski *et al.*, 1983) is likely to reflect certain global conditions, such factors are not considered in the present study, though they must have played an important role in some localities.

Table II also includes a possible extent of the isotope fractionation given in the literature for each factor. Though an additive property of these fractionation extents is by no means proved, it would be basically correct to sum up all these extents to estimate an overall change of the carbon isotopic composition through sedimentary

TABLE II

Factors that may affect $\delta^{13}C$

	Factor	Fractionation (‰)	Reference
PHOTOSYNTHETIC GROWTH	salinity	0	Wong and Sackett, 1978
	temperature	0.36 ~ −0.13/°C −0.23/°C	Wong and Sackett, 1978 Sackett *et al.*, 1965
	aggregation	1 ~ 10 for algal mats	Barghoorn *et al.*, 1977
	partial pressure of CO_2	X	Present study.
DEGRADATION		-3 ± 1	Wada and Parker, 1982
DIAGENESIS		$\gtrsim 0$	Peters *et al.*, 1981

rock formation. The overall change of $\delta^{13}C$ (i.e., $\delta^{13}C_{org/rock} - \delta^{13}C_{source}$), therefore, would be as follows:

$$\begin{aligned}&\delta^{13}C_{org/rock} - \delta^{13}C_{source}\\&= \text{(basic fractionation during algal growth from a carbon source)}\\&+ \text{(deviation caused by change in } CO_2 \text{ partial pressure)}\\&+ \text{(salinity effect)} + \text{(temperature effect)}\\&+ \text{(mat effect)} + \text{(fractionation during degradation)}\\&+ \text{(fractionation during diagenesis)}\end{aligned} \quad (1)$$

Table II shows that the right hand side of Equation (1) is equal to $F_{source} + X - 3 \pm 1 \pm 0.36 \cdot \Delta t + (1 \sim 10)$, where F_{source} represents the fractionation from a carbon source to algal organic carbon under normal growth conditions. X represents the deviation of the fractionation under a certain CO_2 partial pressure, and Δt stands for the difference of Precambrian temperature from the one employed for laboratory cultures.

In order to introduce the result from Precambrian rocks, Equation (1) should be modified as follows:

$$\begin{aligned}&\delta^{13}C_{org/rock} - \delta^{13}C_{carb/rock}\\&= \delta + F_{source} + X - 3 \pm 1 \pm 0.36 \cdot \Delta t + (1 \sim 10),\end{aligned} \quad (2)$$

where δ is the fractionation factor from carbonate to the source:

$$\delta^{13}C_{source} = \delta^{13}C_{carb/rock} + \delta; \quad (3)$$

$\delta = 0$, if the source is bicarbonate, and $\delta = \delta'$ ($\approx -7‰$), if the source is carbon dioxide (Deuser and Degens, 1967).

The overall change, or the difference between $\delta^{13}C_{org/rock}$ and $\delta^{13}C_{carb/rock}$ of Equation (2), must amount to –25.6‰, if the Precambrian reduced carbon is from the algal photosynthetic carbon fixation. Since the temperature of the Precambrian hydrosphere where the biological fixation took place is unknown, let us assume for the moment that there was $\pm$ 10 C° temperature deviation from the temperature at which we cultured algae in laboratory.

Then, the following equation will be obtained for the case where the source of carbon is bicarbonate (i.e., $\delta = 0$):

$$F_{bica} + X = -22.6 \pm 4.6 - (1 \sim 10) \quad (4)$$

If the source of carbon is carbon dioxide, the fractionation from carbonate to carbon dioxide must be taken into account. In this case, the Equation (5) results:

$$F_{CO_2} + X = -\delta' - 22.6 \pm 4.6 - (1 \sim 10). \quad (5)$$

In the present laboratory culture experiments, a variety of mixtures of air and carbon dioxide whose $\delta^{13}C$ had been known was bubbled through the culture flask, and the alga was grown with almost no mat formation. Therefore, the $\delta^{13}C$'s of the feed gas and of the algal body that was grown with virtually no aggregation were what

can be known experimentally, and the difference betwen these experimentally measurable values is related to the fractionation factors given in Table II in the following manner:

$$\delta^{13}C_{org/alga} - \delta^{13}C_{source} = F_{source} + X \tag{6}$$

If the alga uses dissolved bicarbonate, Equation (6) becomes:

$$\delta^{13}C_{org/alga} - \delta^{13}C_{bica} = F_{bica} + X. \tag{7}$$

The left hand side of Equation (7) is not what can be obtained directly from the culture experiment, and a conversion from $\delta^{13}C_{bica}$ to $\delta^{13}C_{CO_2}$ must be done. Using Equation (3) twice, Equation (7) after the conversion will be:

$$\delta^{13}C_{org/alga} - \delta^{13}C_{CO_2} = -\delta' + F_{bica} + X. \tag{8}$$

The left hand side of Equation (8) is what the laboratory culture experiment gives, and $F_{bica} + X$ in the right hand side can be replaced using Equation (4). And the following equation results:

$$\Delta^{13}C = -\delta' - 22.6 \pm 4.6 - (1\sim10), \tag{9}$$

where

$$\Delta^{13}C = \delta^{13}C_{org/alga} - \delta^{13}C_{CO_2}.$$

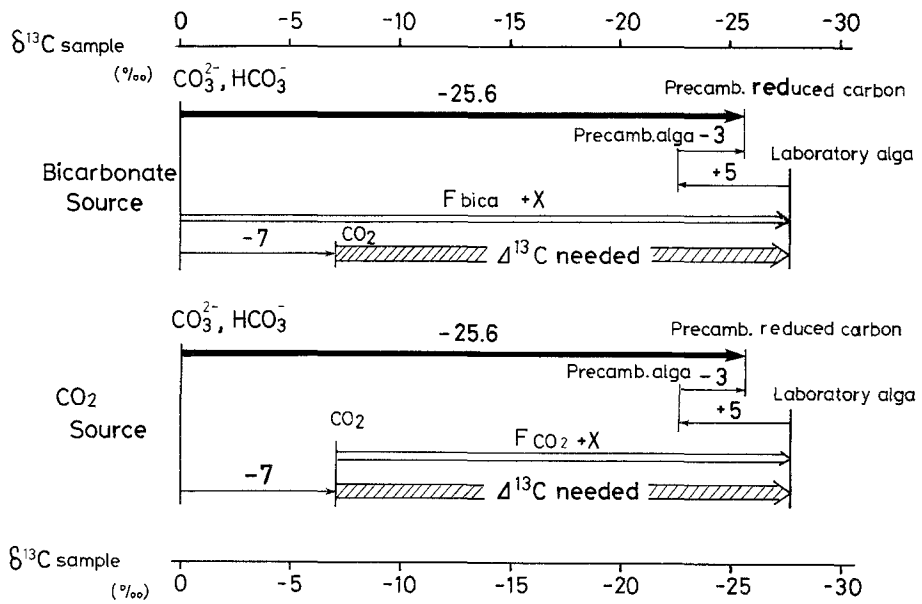

Fig. 1. Relation between $\delta^{13}C$ of Precambrian rocks and $\Delta^{13}C$. For apparent simplicity, the effects of salinity, temperature, and mat formation are combined and considered to be +5‰. The fractionations during degradation and diagnesis are also combined and considered to be −3‰. Note that $\Delta^{13}C$ needed to explain the observed difference between Precambrian reduced carbon and carbonate is the same for both cases: bicarbonate source and carbon dioxide source.

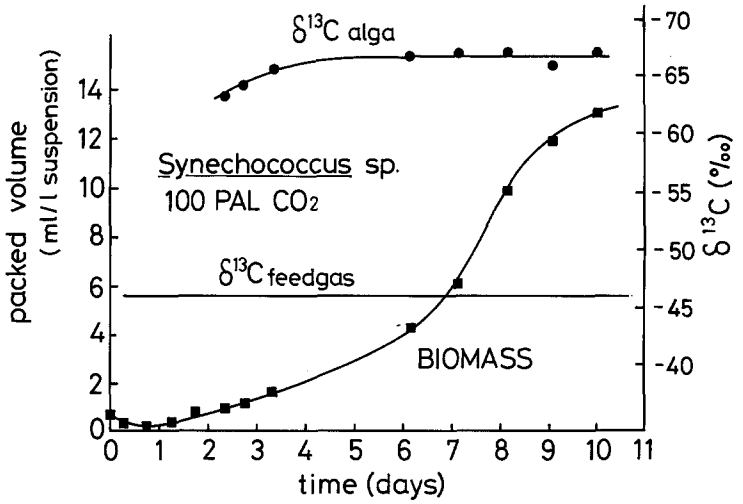

Fig. 2. Variation of $\delta^{13}C$ and the change in biomass during the growth of *Synechococcus* sp.

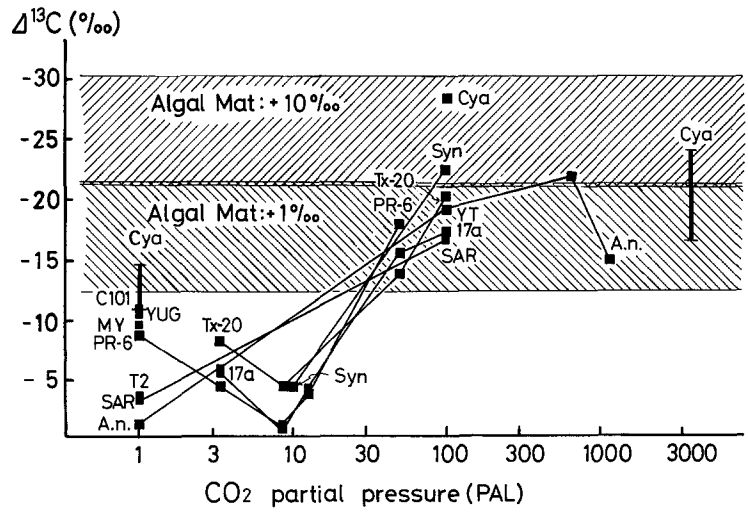

Fig. 3. Effect of CO_2 partial pressure in the feed gas on the fractionation of carbon isotopes by algae. Vertical axis is for the difference of $\delta^{13}C$'s between algal organic matter and the feed gas. The bars for Cya at 1 PAL and at 3333 PAL are from Seckbach and Kaplan (1973). Only a selection of their data is included because of some difficulties in a direct comparison of their result with the present work (see the text). The data for PR–6, Tx–20, and 17a were redrawn from Figure 1 of Calder and Parker (1973). Since their result was not corrected for ^{17}O, it was multiplied by 1.0676 to approximate the ^{17}O correction (Craig, 1957). Though they used a different standard (NBS No. 20) from ours (PDB), the resultant difference in $\Delta^{13}C$ is negligibly small insofar as the redraw is concerned. The algal species they used were as follows: PR–6, *Agmenellum quadruplicatum*; Tx–20, *Anacystis nidulans;* 17a, *Coccochloris elebens*. The results of A.n. and Tx–20 may appear different each other; however, we note that there are several differences between theexperiments such as the culture conditions that might have contributed to the apparent discrepancy.

If the alga uses carbon dioxide as the carbon source move, Equation (6) becomes:

$$\Delta^{13}C = F_{CO_2} + X. \tag{10}$$

In this case, the left hand side is already experimentally obtainable and no conversion is necessary. The substitution of Equation (5) for the right hand side of Equation (10) results in Equation (9).

The argument given here showed that, whatever the source of carbon for algal photosynthesis is, the laboratory culture experiment should satisfy Equation (9) if the combined effects of CO_2 partial pressure and of other factors listed in Table II are to explain the depletion of ^{13}C in Precambrian reduced carbon.

Figure 1 schematically shows how the factors discussed above relate to one another.

4. Results and Discussion

The algal growth and the change of the carbon isotopic composition along time are shown in Figure 2 for the case of *Synechococcus* sp. cultured at 100 PAL (PAL stands for the present atmospheric level and one PAL equals to 0.00030 atm). The $\delta^{13}C_{CO_2}$ of the feed gas was –46.1‰. As can be seen in Figure 2, the carbon isotopic composition remained fairly constant during all but the very early stage of the algal growth. For the rest of this paper, only $\delta^{13}C_{org/alga}$'s obtained from such isotopically stable periods will be presented.

Figure 3 shows the result of our laboratory experiment. The horizontal axis in Figure 3 indicates the CO_2 partial pressure in the feed gas. The vertical axis is for the difference of the carbon isotopic compositions between an alga and the feed gas.

The two slightly overlapping, hatched areas in Figure 3 show the ranges of $\Delta^{13}C$ needed to satisfactorily attribute the cause of the depletion of ^{13}C in Precambrian reduced carbon to CO_2 partial pressure in Precambrian atmosphere. The upper area is the range, when the fractionation of +10‰ is assumed for algal mat formation. The fractionation of +1‰ gives the lower area. These areas are obtained from Equation (9) in the following way.

Equation (9) gives a range which $\Delta^{13}C$ must be within, if the organic carbon in Precambrian sedimentary rocks resulted from algal body. That is:

$$-\delta' - 27.2 - (1\sim10) < \Delta^{13}C < -\delta' - 18.0 - (1\sim10). \tag{11}$$

An approximately –7‰ fractionation from carbonate to carbon dioxide at equilibrium was reported by Deuser and Degens (1967). If the inorganic species of carbon are presumed to be at equilibrium under the present experimental conditions, –7 replaces δ' in expression (11), and expression (12) results:

$$-20.2 - (1\sim10) < \Delta^{13}C < -11.0 - (1\sim10). \tag{12}$$

If the mat effect is +1‰, then $-21.2 < \delta^{13}C < -12.0$ (lower area), and if the effect is +10‰, then $-30.2 < \Delta^{13}C < -21.0$ (upper area).

It is clear from Figure 3 that much of the apparent anomalous depletion of ^{13}C in Precambrian reduced carbon can be understood in terms of the carbon isotope fractionation by algal photosynthetic fixation at high CO_2 partial pressure ranging from about 50 to 500 PAL in Precambrian atmosphere. Figure 3 also includes the result from three algal cultures reported by Calder and Parker (1973) and that from *Cyanidium caldarium* by Seckbach and Kaplan (1973). Their results basically agree with ours so far as the present interest is concerned*.

The earlier argument on $\Delta^{13}C$ showed that the knowledge on chemical form of the carbon source which algae really takes up from the environment is not necessary to determine whether Precambrian reduced carbon is from algal photosynthesis. The reason was schematically given in Figure 1. However, it can be seen from Figure 1 that there exists a very large difference in actual values between $F_{bica} + X$ and $F_{CO_2} + X$ in order to explain the experimental result given in Figure 3: For $\Delta^{13}C = -20$‰, which is about an average of $\Delta^{13}C$ at 100 PAL, $F_{bica} + X$ needs to be –27‰, whereas $F_{CO_2} + X = -20$‰ gives the same $\Delta^{13}C$.

Since the difference in carbon source is only the difference in the form of carbon that passes through algal membrane and is believed not to affect the later enzymatic fixation of carbon through the photosynthetic pathway, actual values of F_{bica} and of F_{CO_2} for algae are expected to be almost the same. Furthermore, the same kind of argument would support the idea that X is also similar among algae. This means in turn that all the algae whose $\delta^{13}C_{org/alga}$ at 100 PAL clusters around –20‰ must be transporting the same form of carbon from surroundings, either bicarbonate or carbon dioxide.

As shown in Figure 3, *Cyanidium caldarium* strain RK–1, an acidophilic alga, cultured in the pH 3 medium gave an unusually large fractionation: $\Delta^{13}C = -28.6$‰ at 100 PAL. This might have resulted from the lack of dissolved bicarbonate in the highly acidic media. The only available carbon source for *C. caldarium* in the media must have been carbon dioxide, and *C. caldarium* might have utilized it directly. If this is the case, $F_{CO_2} + X$ is to be equal to –28.6‰ for the alga. Furthermore, if the fractionation of carbon isotopes at the enzymic fixation of carbon dioxide is independent of the form of carbon that passed through the cell membrane, and if the effect of CO_2 partial pressure for *C. caldarium* is the same for other algae, $\Delta^{13}C = -20$‰ obtained for other algae (see Figure 3) might suggest that they take up bicarbonate instead of carbon dioxide from surroundings as the carbon source for their photosynthesis, since calculated values (Equations (8) and (10)) for their $F_{bica} + X$ and $F_{CO_2} + X$, respectively –27‰ and –20‰, suggest that $F_{bica} + X$ is more likely. This would in turn give support for the assumption that the isotopic equilibrium between bicarbonate and CO_2 exists in the culture media used in the present study.

* The minimum fractionation at about seven PAL of CO_2 partial pressure reported by Calder and Parker is not our current concern and not studied in our present work.

It is well known that the reduced carbon of modern C_3 land plants is isotopically lighter than marine algae ($\delta^{13}C_{org/alga} = -18$ to -20‰) with mean values of $\delta^{13}C_{org/plant}$ ranging from –22‰ to –35‰ (Degens, 1969; and Craig, 1953). From an isotopic geochemical point of view, this difference is considered to be due to the use of bicarbonate for algal photosynthesis and of carbon dioxide for land plants.

Miller and Colman (1980), based on a different approach to the problem, reported an evidence for bicarbonate transport by a blue-green alga, *Coccochloris peniocyctis*.

The present study gave evidence for a higher CO_2 partial pressure in the Precambrian period as well as that for bicarbonate transport by all but one alga examined. It, however, is not the first to propose a higher CO_2 partial pressure from carbon isotopical point of view. Seckbach and Kaplan (1973) grew *Cyandium caldarium* in a pure CO_2 atmosphere and in air, and speculated that the depletion of ^{13}C in Precambrian organic matter may be from algal mats growing at elevated temperatures and under substanstially greater CO_2 partial pressure than at present. A portion of their result is given in Figure 3. It is in a good agreement with our result of *Cyanidium caldarium*; however, their proposition suffers from their experimental set-up that has several weaknesses.

First, it does not fully study the change in carbon isotopic composition along with the algal growth or with the change in CO_2 partial pressure, and consequently raises some questions concerning the applicability of particular values of the $\delta^{13}C$'s presented. Secondly, it does not pay an attention to results produced by difference in the carbon source, focusing only on algae that utilize CO_2 as a sole source of carbon: As discussed earlier, *Cyanidium caldarium* could be quite unique from a carbon isotopic point of view and its $\delta^{13}C$ may be 7‰ lighter than most other algae. Third, *Cyanidium caldarium* is a eukaryotic alga, whose existence earlier than 1.5 billion years ago not known (Schopf, 1978), and, therefore, cannot be responsible for most Precambrian organic carbon. These shortcomings weaken their arguments. However, theirs are not the only one that suffers from such weaknesses.

In particular, the relationship between laboratory obtainable data and those from Precambrian rocks does not appear to have been taken into consideration in any previous work that tried to relate the depletion of ^{13}C to a higher atmospheric CO_2 partial pressure. This resulted in seeking extreme conditions besides higher CO_2 partial pressure so that the carbon isotope fractionation could be maximized. For instance, Pardue *et al.* (1976) reported maximum carbon isotope fractionations from CO_2 to algal organic carbon during the photosynthesis by blue-green algae and a green alga, and tried to compare the result directly with the difference in $\delta^{13}C$'s between Precambrian organic carbon and carbonate. Although they suggested from their study a high carbon dioxide availability for the early photosynthetic organisms, their argument seems to have fallen short of being accepted widely (Towe, 1982), because even the maximum fractionations appeared still marginal by the direct comparison in explaining the depletion and because the conditions for the maximum fractionations were not necessarily very plausible.

The present study is free of the past shortcomings and would put a higher atmospheric CO_2 on a more solid ground as a major cause for the average depletion of ^{13}C in Precambrian reduced carbon.

A high CO_2 partial pressure in Precambrian atmosphere appears plausible from different points of view. Though no solid data is available, it seems generally believed that volcanic activity was high early in the Earth's history. If it is the case, the amount of released CO_2 through volcanic gas into an atmosphere should be larger in the Precambrian period. Tucker (1982) suggested a higher partial pressure of CO_2 in Precambrian times than at present, when he considered the conditions for the Precambrian dolomite formation. Igo (1980) concluded in a review article on Precambrian microfossils that the CO_2 partial pressure in Precambrian atmosphere must have been higher than the present level.

Though these evidences support the idea that the Precambrian atmosphere contained a large amount of CO_2, they do not give what the order of magnitude of the CO_2 partial pressure was. The present study, however, indicates that it could have been two orders of magnitude higher in the Precambrian period than it is now.

This is derived from the average depletion of ^{13}C in Precambrian reduced carbon, and as such, the high CO_2 partial pressure should not be considered as the sole cause for determining the carbon isotopic composition of every Precambrian organic carbon.

Localized deviations of the carbon availability from the global average is known even today. For instance, because of the chemistry particular to certain localities, there are aquatic ecosystems today where the concentration of bicarbonate is much higher than what would be expected from the present partial pressure of atmospheric CO_2. Schoell and Wellmer (1981 and 1982) suggested that a local methane bacterial action might have further depleted ^{13}C in some Precambrian reduced carbon. The depletion of ^{13}C in the stromatolitic limestones of Bulawayan group from Huntsman Quarries, Zimbabwe and of other localities (Eichmann and Schidlowski, 1975), for instance, is a few permils more than what would be expected. This might indicate a higher availability of carbon for the organisms that formed the stromatolitic limestones.

Given the high CO_2 concentration in the Precambrian atmosphere as a global background to work with, it would be of further interest to elucidate the individual causes for the deviations of $\delta^{13}C$'s from the average carbon isotopic composition in the Precambrian period, since it might give a better understanding of the Precambrian environments in which life on the Earth must have originated and lived most of its past.

In conclusion, we would like to suggest from the present study that it is reasonable to assume at this moment that the Precambrian reduced carbon whose $\delta^{13}C_{org/rock}$ clusters around –26‰ is of biological origin, and that the ribulose 1,5-biphosphate carboxylase reaction was responsible for the fixation of carbon from inorganic carbon sources, and that the apparently anomalous ^{13}C depletion could reflect the global condition of a high CO_2 partial pressure (about two orders of magnitude higher than the present level) in the Precambrian atmosphere.

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Note added in proof

In views of current interest in the interaction between the Earth and the biosphere (e.g., Lovelock and Whitfield, 1982, *Nature* **296**, 561), and of the unfortunate fact that theoretical approaches to the history of atmospheric carbon dioxide concentration is yet to find a solid conclusion (see Kuhn and Kasting, 1983, *Nature* **301**, 53), it appears significant that the present experimental study gave a similar conclusion, as far as the carbon dioxide concentration is concerned, to the one given in a theoretical study on the evolution of terrestrial atmosphere (Hart, 1978, *Icarus* **33**, 23).

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