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ANALYSIS OF ISOTOPIC RATIO OF IRON IN ANTARCTIC LICHENS, *USNEA SPHACELATA* AND *UMBILICARIA APRINA*

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Abstract: For the purpose of getting information about the mechanism of uptake of iron by lichens, isotopic distribution of iron in two kinds of lichens, *Umbilicaria aprina* and *Usnea sphacelata* in the vicinity of Syowa Station, Antarctica, was studied. Lichen samples were tentatively separated into two categorical sites, active growing site and inactive site.

As the first attempt, the isotopic ratio of ^{54}Fe to ^{58}Fe incorporated in the lichens was determined by neutron activation method and partly by ICP mass technique. The isotopic ratios ($^{54}Fe/^{58}Fe$) of the active growing site were significantly smaller than those of the inactive site or substratum for both lichens. An autonomous incorporation of iron with a kinetic isotope effect by these lichens can be considered.

1. Introduction

There has been an increasing amount of data concerning the uptake of many mineral elements by lichens. Some of them provide a clue for utilization of lichens as markers of mineral distribution patterns or as monitors of ecological surveillances. Since many reviews and articles have already been published, some recent articles and the references in them provide a brief history of the study in this field (BROWN, 1976, 1987). Among the mineral elements, the effect of heavy metals on lichen activity is a subject of special interest to many lichenologists. For instance, the activity of photosynthesis in lichen is substantially depressed by the addition of heavy metals (BROWN and BECKETT, 1983), although some lichens are still frequently found at mining sites which are contaminated with heavy metals.

In some lichens, iron is incorporated in an abnormally large amount compared with other heavy metals such as copper, zinc and manganese, and its incorporation is comparable with that of substratum. Our main purpose of this work is to get some information about whether or not lichens incorporate heavy metal such as iron autonomously. In other words, the question whether iron incorporated by lichens is an essential necessity to their metabolism might find an answer in the analysis of the distribution pattern of stable isotopes of iron.

Iron has four stable isotopes, ^{54}Fe(5.8%), ^{56}Fe(91.7%), ^{57}Fe(2.2%) and ^{58}Fe(0.3%).

Natural abundances of iron shown in the parentheses vary from data to data (IUPAC SUBCOMMITTEE, 1984). In order to diminish the artificial ambiguities in the variance, Antarctic lichens can be regarded as reliable standards for the analysis of isotopic ratios of iron as well as other elements in lichens. We selected *Umbilicaria aprina* and *Usnea sphacelata*, because both genera are distributed almost all over the world including Antarctica and would serve as a standard.

2. Experimental

2.1. Lichen samples

Lichens were collected from the region near Syowa Station. *Umbilicaria aprina*: *ca.* 50 m above sea level at Mukai Rocks, Sôya Coast, Enderby Land, coll. M. INOUE no. 18320, May 3, 1986. *Usnea sphacelata*: *ca.* 80 m above sea level at Cape Hinode, Prince Olav Coast, Enderby Land, coll. M. INOUE no. 18502, Sept. 17, 1986.

U. aprina was 7.5 cm in diameter of thallus and 2.5 g in dry form. *U. sphacelata* was 5 cm long. Both lichens inhabited the surface of gneiss and their longevities can be estimated as several hundred years.

The thallus of dried *U. aprina* was cut into four groups of strips (0.5 cm wide); the outermost strip, inner three strips, the innermost two strips, and the rest including rhizines. Among these strips, the outermost strip and the innermost two strips were submitted to the analysis, as they seemed to represent the two extremes of physiological activities, *e.g.*, active site and inactive site of metabolism, respectively.

In the case of *U. sphacelata*, about a hundred of thallus were cut into four groups of small pieces of strings; upper part is colored dark green, upper-middle part green and yellow, lower-middle part pale yellow, and bottom part including rhizines and substratum. Upper part, active site, and lower-middle part, inactive site of thallus of *U. sphacelata* were submitted to the analysis.

The substrata of both lichens, which are gneisses of different appearance, were also analyzed. Both gneisses were cleaved into plates of *ca.* 1 to 2 cm thickness. Gneiss of *U. aprina* was harder than that of *U. sphacelata* which was easily broken even by hand. In the gneiss of *U. aprina*, quartz and feldspar were observed clearly under a magnifying glass in addition to black mica which occurred mainly as a single layer. The degree of metamorphism and weathering seemed to be different between the two substrata.

2.2. Sample preparation

Although the chemical nature of iron incorporated in lichen is not yet clearly elucidated, we attempted preliminarily successive extractions of iron by leaching with aqueous media of different acidity; deionized water, acetic acid and nitric acid. By leaching with acidic solutions, iron which is physico-chemically bound with lichen tissue or weakly chemically bound with organic substances could be separated depending on the acidity of media employed. Iron with more strongly incorporated in lichen will remain in the residue.

About 0.5 g of dry lichen and ground substrata samples were successively treated with deionized water, 0.4 M acetic acid and 3.5 M nitric acid in a 20 m*l* sintered glass tube

(No. 3). Each treatment was performed twice with 10 m*l* of leaching solution for 30 min. Those solutions as well as the residue were digested in the Teflon beaker using HNO_3, $HClO_4$ and HF as is the usual manner. One-third of each digested solution was submitted to the analysis of iron content. The rest was submitted to the selective extraction of Fe(III) with *N*-benzyl-*N*-phenylhydroxyl amine in chloroform (SHENDRIKAR, 1969). From the organic layer, iron was reversely extracted into the acidic aqueous solution (pH 0.5), to be precipitated in the form of $Fe(OH)_3$ by the addition of aqueous ammonia. The precipitate was then collected on the filter paper which was repeatedly washed with nitric acid to remove iron contamination. The precipitate was dried in the desiccator and was shielded in a quartz tube (Viosil F) including the filter paper for the neutron activation analysis.

2.3. Neutron activation analysis

Usually, analysis of isotopic abundance of an element is performed by mass spectrometry. According to our trial use of mass spectrometry for isotopic analysis, any stable ion beam of iron was not obtained. By using double Faraday cup detector, mass peak at ^{56}Fe was detected in the range of 700 to 1500 mV with the use of either single or double filament ionization techniques. However, the ion beam was not stable enough to integrate ion beam currents of four isotopes of iron. The recent experiment of isotopic abundance of ^{58}Fe was done by neutron activation method by using highly enriched Fe_2O_3 (73.26% ^{58}Fe) to give a value of 0.280% (JAMES and CARNI, 1980). This method, however, is not adequate to routine analysis of biological samples.

Neutron activation analysis was carried out at Japan Atomic Energy Research Institute (JAERI) in Tokai-mura using JRR-4 atomic reactor. The variance in the ratio of ^{54}Fe to ^{58}Fe of the sample shielded in the quartz tube (6 h irradiation in S pipe) was determined by measuring the gamma-ray at 834.8 keV ($^{54}Fe \xrightarrow{(n,p)} {}^{54}Mn$, 100% emission, $t_{1/2}=312.5$ d) and 1099.3 keV ($^{58}Fe \xrightarrow{(n,\gamma)} {}^{59}Fe$, 56.5% emission, $t_{1/2}=44.6$ d) for 3000 s after cooling period of 45 days. For the ratio of ^{57}Fe and ^{58}Fe, the iron sample in the quartz tube was seamed into a polyethylene bag again after a prolonged period of cooling, and the same gamma-ray spectrometry at 122.1 keV ($^{57}Fe \xrightarrow{(n,p)} {}^{57}Mn$, 10.8% emission, $t_{1/2}=$ 1.75 min) and 810.8 keV ($^{58}Fe \xrightarrow{(n,p)} {}^{58}Mn$, 88.2% emission, $t_{1/2}=1.09$ min) was carried out for 200 s immediately after 30 s irradiation (pneumatic tube). Gamma-ray spectrometry was performed using a Ge(Li) detector with 4000 channel of 0.5 keV/ch resolution.

Because the ratios of gamma-ray intensities, 843.8 keV to 1099.3 keV and 122.1 keV to 810.8 keV, can be considered to be strictly related with the amounts of corresponding isotopes, ^{54}Fe to ^{58}Fe and ^{57}Fe to ^{58}Fe, no standard substance is necessary to obtain the information of isotopic ratio. An unavoidable inadequacy of this method is a lack of data concerning with the cross section of (n, p) nuclear reactions of nuclides monitored. Observed value is, therefore, not the absolute ratio of isotopes but the variance in the relative ratio. Possible interferences from the quartz tube and the filter paper were not observed in the region of gamma-ray energy monitored. Detailed discussions about the quartz tube used in this experiment, Viosil-F, has been made by YONEZAWA and HOSHI (1990).

3. Results and Discussion

3.1. Element analysis

Simultaneous multi-element analysis by inductively coupled plasma (ICP) spectrometry was performed for the lichens and their substrata. The results are summarized in Table 1. Because of the limited amount of lichen, the samples used for the element analysis were not the same samples as used for the isotopic determination but the one growing on the same substrata. A preliminary analysis of lichen and substratum showed some variations in iron contents from sample to sample, showing an inhomogeneity of the distribution of elements in the sample. However, we could see the gross pattern of incorporation of mineral elements by these lichens. In some elements, such as Mn, Cr, Cu and Zn, there seemed to exist no significant differences in their content between lichen and substratum. These data might induce the idea that iron in these lichens has originated from the trapped substrate particles in lichen. The molar ratios of Fe to Ti and Fe to Al in these lichens, however, were clearly different from those in corresponding substata (Table 2). The molar ratio of Al to Ti also shows no relation between lichen and substratum. Therefore, it could be concluded that iron is incorporated in these lichens. Some basic arguments on these points have been made by several authors (NIEBOER and TOMASSINI, 1978; BROWN and BECKETT, 1984; PUCKETT, 1985). Titanium was found in both lichens as one of the incorporated transition elements in a considerable amount second to iron; 183 μg/g in *U. aprina* and 108 μg/g in *U. sphacelata*

Table 1. Element analysis of lichens and their substrata by ICP spectrometry (μg/g).

Element	Sample			
	U. aprina	Substratum (gneiss)	*U. sphacelata*	Substratum (gneiss)
Al	940	39890	410	23360
B	53	1816	34	1414
Ca	890	4610	1060	2480
Cu	24	26	28	23
Fe	2060	3180	910	5410
K	1640	26240	1750	15840
Li	19	25	19	26
Mg	970	1000	1240	1960
Mn	24	55	16	45
Mo	3	4	4	4
Na	1350	21330	800	20610
Ni	14	15	12	64
P	450	780	310	140
Pb	20	39	27	32
Sr	6	34	5	25
V	6.8	10.0	5.1	29.3
Zn	27	29	32	117
Cr	26	30	24	49
Ti	183	1367	108	1740
Zr	4	31	4	80

(13 and 6% of the respective substrata) (Table 1). And the molar ratios of Al to Ti in these lichens were smaller than those in the substrata (Table 2). These findings suggest concentration of titanium by these lichens. PAIS (1983) reported that titanium ascorbate stimulates the growth of some vascular plants. Although neither the necessity of titanium to the plant activity nor the chemical mechanism of physiological activity of titanium is clear at this moment, titanium might contribute to the biological activities in these lichens.

The differences between the two gneisses in the contents of certain elements such as Al, Ca, Fe, K, Mg, Ni, Ti and Zr, would be explained by the difference in metamorphism. Further investigation on metamorphism of these gneisses was not carried out.

The content of iron in leaching solutions in the discrimination process is summarized in Table 3, according to ICP mass spectrometry on ^{56}Fe. As only trace amounts of iron (less than 0.5% of total amount) were detected in the leaching solutions by deionized water, the data were omitted from the table. From active and inactive sites of both lichens, more than half of the iron was eluted by acetic and nitric acids. Strongly bound iron which was not eluted by nitric acid remained in the residue. The amount of iron eluted by acetic acid was smaller than by nitric acid. The amounts of iron eluted by acetic and nitric acids were in reverse order to those from active and inactive sites of both lichens; the amount of iron in the inactive site eluted by acetic acid was roughly twice that in the active site, and iron in the active site eluted by nitric acid was greater than that in the inactive site.

Table 2. Molar ratios of Fe to Al and Fe to Ti of U. aprina, U. sphacelata *and their substrata.*

Molar ratio	*U. aprina*		*U. sphacelata*	
	Thallus	Substratum	Thallus	Substratum
Fe/Al	2.2	0.08	2.2	0.23
Fe/Ti	11.3	2.3	8.4	3.1
Al/Ti	5.1	29.2	3.8	13.4

Table 3. Content of iron in leaching solution of lichens and their substrate by ICP mass spectrometry (μg/g).

Sample	Total	Leaching media		
		Acetic acid	Nitric acid	Residue
U. aprina				
Active site	2270	186(8.3%)	1084(48.4%)	970(43.3%)
Inactive site	1557	245(15.7%)	609(39.1%)	703(45.2%)
Substratum (gneiss)	2829	394(13.9%)	1151(40.7%)	1284(45.4%)
U. sphacelata				
Active site	790	90(11.4%)	444(56.2%)	256(32.4%)
Inactive site	815	161(19.8%)	262(32.1%)	392(48.1%)
Substratum (gneiss)	5753	927(16.1%)	78(1.4%)	4748(82.5%)

It is well known that many lichens produce a variety of phenolic substances as well as carboxylic acids as secondary metabolites on their fungal hyphae. As acetic acid has low affinity in interaction with Fe(II) or Fe(III), the binding strength of iron eluted by acetic acid would be comparable to or less than that of physico-chemical interaction such as iron-exchange. Complex formation of iron with secondary metabolic substances produced at mycobiont such as usnic, norstictic and gylophoric acids, might be affected as one of the incorporation mechanisms of iron by these lichens. Many kinds of phenolic compounds are immediately reminiscent of a color reaction with Fe(III) to produce hexaphenoxido iron (III) complex which is easily decomposed by strong acid, such as 3.5 M nitric acid (SOLOWAY and WILEN, 1952). It is well established that green alga is observed all over the inner layer in these lichens with the same extent of distribution. As the high ability of green algae in uptaking iron is also well known, the iron in the residual parts could be incorporated in the algal cells of these lichens which have high resistance against nitric acid.

The elution patterns of iron in two kinds of gneisses were different from each other. Nitric acid eluted iron in only 1.4% of the total amount from substratum of *U. sphacelata*, which stands in contrast with that of *U. aprina*. More interestingly, around 15% of total iron was eluted by acetic acid in both gneisses. The unexpected fairly large amounts of iron eluted by acetic acid in both substrata might be related to the incorporation of iron by endolithic microorganisms (FRIEDMANN, 1982). Although it would be hard to determine the degree of metamorphism quantitatively from the mineralogical point of view, these data would be explained by the differences between these gneisses in degrees of metamorphism or weathering by endolithic microorganisms.

3.2. Isotopic analysis

The isotopic ratios of ^{54}Fe to ^{58}Fe by neutron activation method are summarized in Table 4. Despite of smaller cross section of (n, p) nuclear reaction of ^{54}Fe than that of (n, γ) reaction of ^{58}Fe, ^{54}Mn and ^{59}Fe were clearly observed in the spectrum chart. The net counts of ^{54}Mn (834.8 keV) and ^{59}Fe (1099.3 keV) were in the ranges of 390 to 650 and 2300 to 3900, respectively; the corresponding relative standard deviations were in the ranges of 10 to 7% and 3 to 2%, respectively. For the samples eluted by acetic acid, however, gamma-ray peak at 834.8 keV had not enough intensity. The ratio of ^{57}Fe to ^{58}Fe was not obtained, because of low flux of fast neutron in the pneumatic apparatus and short irradiation time. For the samples of *U. sphacelata*, additional data of these ratios obtained by ICP mass spectrometry are also listed in the table as a comparator. In the case of the ICP mass data, the standard deviations were more fluctuating than in neutron activation, probably depending on the stability of plasma flame. According to the data of IUPAC, isotopic ratios of ^{54}Fe to ^{58}Fe and ^{57}Fe to ^{58}Fe of natural abundance can be calculated in two digits, *e.g.* 20(.7) and 2.6(4) respectively, which are roughly the same values as those found in the inactive site or substratum of *U. sphacelata*. Although from the data of *U. sphacelata* there exist some discrepancies in the order of values between the two methods, the ratio of cross section of (n, p) reaction of ^{58}Fe and (n, γ) reaction of ^{54}Fe can be calculated to be roughly 900 to 1000. It would be concluded that smaller values of the isotopic ratio of ^{54}Fe to ^{58}Fe are always observed in the samples of active sites of both lichens. The same decreasing

Table 4. Isotopic ratios of ^{54}Fe to ^{58}Fe and ^{54}Fe to ^{57}Fe obtained by neutron activation method and ICP mass spectrometry.

Samples	Neutron activation	ICP mass	
	Relative intensity $^{54}Fe/^{58}Fe$ (in $\sigma\,^{58}Fe_{(n,\gamma)}/\sigma\,^{54}Fe_{(n,p)}$)*	Isotopic ratio	
		$^{54}Fe/^{58}Fe$	$^{54}Fe/^{57}Fe$
U. aprina			
Active site			
residual	0.0168±0.0015	—	—
nitric acid	0.0158±0.0016	—	—
acetic acid	—	—	—
Inactive site			
residual	0.0203±0.0018	—	—
nitric acid	0.0188±0.0019	—	—
acetic acid	—	—	—
Substratum			
residual	0.0245±0.0022	—	—
nitric acid	0.0237±0.0018	—	—
acetic acid	—	—	—
U. sphacelata			
Active site			
residual	0.0164±0.0015	16.5±1.9	2.36±0.17
nitric acid	0.0182±0.0018	14.0±0.6	2.31±0.02
acetic acid	—	14.3±0.4	2.18±0.09
Inactive site			
residual	0.0214±0.0021	19.6±1.3	2.63±0.18
nitric acid	0.0224±0.0025	21.0±2.1	2.50±0.25
acetic acid	—	19.1±2.4	2.51±0.32
Substratum			
residual	0.0244±0.0019	19.4±1.2	2.80±0.18
nitric acid	0.0230±0.0018	20.4±1.7	2.80±0.26
acetic acid	—	19.4±1.8	2.65±0.49

* Represented in the unit of a radio of cross section of (n, γ) nuclear reaction to that of (n, p) nuclear reaction which is unknown.

pattern of ^{54}Fe to ^{57}Fe can be seen in *U. sphacelata*. These findings lead to the conclusive evidence that heavier isotope of iron is enriched in these lichens at active growing site.

If incorporation of iron by lichen is influenced by a simple physico-chemical interaction, any isotope effect would not be observed, because the isotopically enriched or stripped element by a weak interaction such as thermodynamic ion-exchange will be dispersed into the environment with excess amount of protic media, like water, during a long period of time. Since the difference between the mass of ^{54}Fe and ^{58}Fe can only provide a small difference in the energy of vibrational ground state of chemical bond of iron incorporated by lichen, a very small isotope effect would be expected by a thermodynamic equilibrium mechanism. Therefore, an alternative mechanism, *e.g.*, a kinetic isotope effect, must be considered. It could be deduced that these lichens incorporate iron autonomously by a kinetic mechanism implemented probably in the

secondary metabolic process which is more intensifying in the acitve site than in the inactive site. According to the data of activation analysis, the abundance of ^{58}Fe at the active site in both lichens is about 1.4 times abundance of substratum. This rather overestimated value of enrichment would be accepted when the great longevities of the both lichens are considered.

In the four isotopes of iron, only ^{57}Fe ($I=1/2$) can be categorized in the magnetic isotope effect in addition to the ordinary thermodynamic and kinetic isotope effects (MOLIN, 1984). This is one of the reasons why we selected lichens of Antarctica. The strength of terrestrial magnetism in Antarctica is higher than that in other places except the North Pole region. Terrestrial magnetism around Syowa Station is 0.05 nT. The efficiency of magnetic isotope effect of some photochemically induced organic reactions can reach a maximum point at around 0.15 T (TURRO *et al.*, 1981). No obvious difference between the ratios of ^{54}Fe to ^{57}Fe and ^{54}Fe to ^{58}Fe could be concluded from the data of ICP mass. More accuracy in the data will be needed to discuss about the magnetic isotope effect.

4. Concluding Remarks

The isotope effect of iron in two kinds of lichens was presented as the first example in the natural environments. The neturon activation analysis for the determination of isotopic ratio can be a useful tool for qualitative comparison; by choosing nuclides of similar length of lifetime produced by either (n, γ) or (n, p) nuclear reactions, differences in the isotopic ratios can be compared with those of environment. Some isotopic ratios to which neutron activation is applicable are $^{47}Ti/^{48}Ti$, $^{106}Cd/^{111}Cd$, $^{112}Sn/^{122}Sn$ and so forth (by analyzing the ratios of ^{47}Sc ($t_{1/2}=3.42$ d)/^{48}Sc ($t_{1/2}=1.82$ d), ^{106}Ag ($t_{1/2}=8.3$ d)/^{111}Ag ($t_{1/2}=7.5$ d), ^{113}Sn ($t_{1/2}=115.1$ d)/^{123}Sn ($t_{1/2}=129.2$ d) and so on, respectively). In addition to the nondestructive character of this method, one can point out the sensitivity against the isotope of very small natural abundance, like ^{58}Fe (0.3%). We are now studying the applicability of neutron activation method to the quantitative assessment of isotope effect as well as a further investigation of isotope enrichment by lichens.

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