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Original Article

Accumulation of iron and magnesium in growing Hijiki (*Sargassum fusiforme*†) plants.

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Summary

Hijiki (*Sargassum fusiforme*) plants were collected during their growth period from the end of November through April on the sea coast of Kushimoto, Wakayama, Japan. The samples were brought back to the laboratory under ice-cold conditions and washed thoroughly with artificial sea-water and distilled water successively. The plants were cut into pieces of a 10 cm length along the stalk, and separated into leaves and stalks. The samples were lyophilized, and decomposed in a mixture of conc H_2SO_4 and conc HNO_3 (3 to 1, v/v) on an electric heater. Iron (*Fe*) and magnesium (*Mg*) in 1N HCl were determined with an atomic absorption spectrophotometer.

Fe accumulated in the respective tissues at concentrations of 70 to 110 μg/g dry weight of tissues at the beginning of growth and *Mg*, at concentrations of 7 to 9 mg/g dry weight of tissues. *Fe* accumulated at 20 to 300 μg/g dry weight through the growing period until the beginning of April. *Mg* accumulated at 5 to 30 mg/g dry weight of tissues through the growing period. *Mg* accumulation seemed to be more than enough for its biochemically required concentrations in the tissues of Hijiki.

Keywords: atomic absorption spectrophotometry; growing Hijiki; Hijiki, *Sargassum fusiforme*; Iron (*Fe*); magnesium (*Mg*); seaweed.

Introduction

Hijiki (*Sargassum fusiforme*) is rich in some nutritionally beneficial minerals[2, 3] and it has high contents of dietary fibers[4]. Hijiki, a family of Brown algae, grows on rocks on the sea-coast of Japan, bathed by the Kuroshio Current stream. Thus, Hijiki is familiar and used as a traditional food-stuff in daily Japanese dishes[5].

In the previous paper[6], we reported on the accumulation of manganese (*Mn*) and zinc (*Zn*) during the growth of Hijiki plants. From the plant-physiological view point, it is conceivable that *Mn* works as an essential element for the polysaccharide biosynthesis and *Zn* as an element essential to carbonate dehydratase in the carbon dioxide assimilating process.

Magnesium (*Mg*) and iron (*Fe*) are essential elements for the photosynthetic activity and electron transfer in the cells. For maintenance of those functions, *Mg* and *Fe* accumulation may occur as fundamental processes. We intended to elucidate their accumulation processes during the growth of Hijiki plants.

Materials and Methods

1. Sampling of Hijiki plants

Hijiki [*Sargassum fusiforme*, (Harvey) Setchell*] plants harvested on the sea-coast of Kushimoto, Wakayama, Japan, were used. The embryos of Hijiki are fixed on rocks and germinate in summer[7-9]. At the beginning of winter, they grow to the primary-leaf stage through the germlings in autumn. Early samples corresponding to this stage were collected in November. After then, Hijiki grows to become adult plants.

All the samples were collected at the time of the lowest tide from the end of November through April on a fixed rock.

The harvested plant samples were packed in an ice-cold box and brought back to the laboratory on the same day.

2. Fractionation of Hijiki plants.

The sample plants were washed thoroughly with artificial sea water (three times) and distilled water[10] (three times), successively. The samples, after blotted with filter paper to remove extra water, were cut to pieces of a 10 cm

† Newly proposed taxonomic name of *Hizikia fusiforme* Okam.[1]

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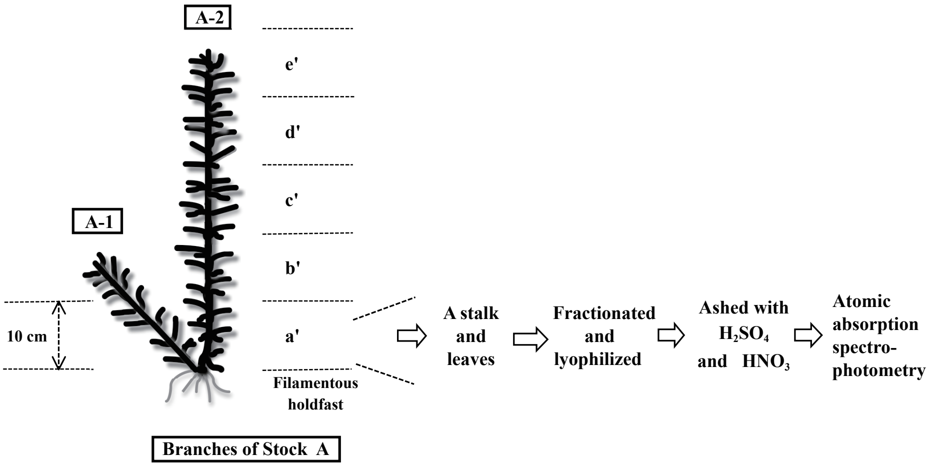

Fig. 1 Fractionation of Hijiki plants.

After washing, the sample plants were cut into pieces of a 10 cm length, and separated into respective tissues, a stalk and leaves. Their respective sections were designated as a', b', c' etc from the lower end to the upper end. After lyophilized, the respective specimens were ashed with a mixture of conc H_2SO_4 and conc HNO_3, and dissolved in 0.1 N HCl to determine their iron and magnesium contents by atomic absorption spectrophotometry.

length from the lower end to the top, and each piece was designated as a', b', c', etc from the lower end to the upper end. They were separated to stalks and leaves (Fig. 1), and placed in small polyethylene bags, stored frozen under −30 ℃, and lyophilized.

3. Ashing the samples

Respective specimens were decomposed in a mixture of conc H_2SO_4 and conc HNO_3 (3 to 1, v/v) on an electric heater for a few hours.

4. Atomic absorption spectrophotometry

The decomposed samples were dissolved in 1 N HCl, and *Fe* and *Mg* were determined by using the Hollow-Cathode lamps for iron and magnesium (Hamamatsu Photonics Co. ltd.) with an atomic absorption spectrophotometer (Shimadzu AA-6200, Japan).

5. Reagents

The reagents were of the JIS Special Grade or its equivalent.

Iron Standard Solution (JCSS) contained 1,000 mg *Fe*/L as $Fe(NO_3)_2$ in 0.1 N HNO_3. Magnesium Standard Solution (JCSS) contained 1,000 mg *Mg*/L as $Mg(NO_3)_2$ in 0.1 N HNO_3.

Results

1. Growth of the Hijiki plants (Table 1)

1-1. The Hijiki plants, harvested at the end of November, possessed 6 to 13 leaves, 2.13 ± 1.13 cm in length, in a stock, corresponding to the primary leaf stage. The water contents of the fresh leaves, mostly 88%, were several percent lower than those of the samples harvested in February, suggesting that they were in a rather mature state.

1-2. In February, the Hijiki stalks grew to 12–17 cm in length, showing the shape of an adult plant. The water contents in the respective sections were 85 to 88% of the stalk fresh weight and 90 to 91% of the leaf fresh weight. These trends were observed in the samples harvested in March and April (Table 1). The leaves of the upper sections had higher contents of water than those of the lower sections, reflecting their immaturity. The water contents of the leaves were higher than those of the stalks in the same sections, suggesting that immature leaves co-exist among these leaves, especially in the upper sections.

2. Accumulation of *Fe* (Table 2)

2-1. In the Hijiki plants of the primary-leaf stage harvested in November, the iron contents were 70 to 110 μg *Fe*/g dry tissues, corresponding to 1.2 to 2 micromoles *Fe*/g dry weight of the tissues.

2-2. The leaves harvested in February showed lower *Fe* contents than those harvested in March and April. In most branches, iron concentrations in the leaves harvested in April increased somewhat from those harvested in March. In most branches, the leaves of the lowest sections, designated as a' or the highest sections designated as c' or d' in Table 2, showed highest iron concentrations in a branch.

2-3. In the stalks, the concentration of iron was often rather lower than those of the leaves in the same sections of a branch. It is remarkable that the fluctuation of the

Table 1 Water contents in the Hijiki plants during growth.

Date of harvest	Samples*	Tissues**	Number of leaves	Wet weight (g)	Dry weight (g)	Water contents (g water/g wet weight of tissues)
11/30/2008	A	Leaves	13	1.6187	0.1759	0.891
	B		9	1.0672	0.1174	0.890
	E		7	0.4927	0.0612	0.876
	F		12	0.5906	0.0734	0.876
	K		6	0.4844	0.0543	0.888

Date of harvest	Samples*	Tissues	Sections***	Wet weight (g)	Dry weight (g)	Water contents (g water / g wet weight of tissues)
2/8/2009	A-1	Stalks	c'	0.0688	0.0104	0.849
			b'	0.6070	0.0850	0.860
			a'	0.4863	0.0738	0.848
	A-2	Stalks	b'	0.1865	0.0212	0.886
			a'	0.3741	0.0485	0.870
	A-3	Stalks	b'	0.2837	0.0342	0.880
			a'	0.4080	0.0500	0.877
	A-4	Stalks	b'	0.1139	0.0134	0.883
			a'	0.3808	0.0466	0.878
	A-1	Leaves	c'	1.1991	0.1146	0.904
			b'	2.1814	0.2354	0.892
			a'	1.0568	0.1047	0.901
	A-2	Leaves	b'	1.1957	0.1142	0.904
			a'	0.6428	0.0606	0.906
	A-3	Leaves	b'	1.1504	0.1095	0.905
			a'	0.6423	0.0588	0.908
	A-4	Leaves	b'	0.5573	0.0525	0.906
			a'	0.5837	0.0552	0.905

Date of harvest	Samples*	Tissues	Sections***	Wet weight (g)	Dry weight (g)	Water contents (g water / g wet weight of tissues)
3/11/2009	A-1	Stalks	d'	0.5369	0.0801	0.851
			c'	0.7266	0.1213	0.833
			b'	0.7363	0.1282	0.826
			a'	0.5749	0.1050	0.817
	A-2	Stalks	d'	0.4166	0.0587	0.859
			c'	0.7172	0.1130	0.842
			b'	0.7184	0.1219	0.830
			a'	0.5897	0.0992	0.832
	A-3	Stalks	d'	0.0742	0.0102	0.863
			c'	0.6088	0.0883	0.855
			b'	0.6382	0.0942	0.852
			a'	0.5203	0.0845	0.838
	A-1	Leaves	d'	4.5252	0.4458	0.901
			c'	2.7554	0.3028	0.890
			b'	2.4778	0.2658	0.893
			a'	0.7324	0.0766	0.895
	A-2	Leaves	d'	3.6957	0.3680	0.900
			c'	2.5382	0.2788	0.890
			b'	2.5854	0.2720	0.895
			a'	0.2301	0.0264	0.885
	A-3	Leaves	d'	0.7658	0.0699	0.909
			c'	2.7152	0.2766	0.898
			b'	1.6423	0.1652	0.899
			a'	0.6545	0.0702	0.893

Date of harvest	Samples*	Tissues	Sections***	Wet weight (g)	Dry weight (g)	Water contents (g water / g wet weight of tissues)
4/12/2009	A-1	Stalks	e'	0.7400	0.0980	0.868
			d'	1.0452	0.1534	0.853
			c'	1.0735	0.1858	0.827
			b'	1.9837	0.1923	0.903
			a'	0.7316	0.1353	0.815
	A-2	Stalks	d'	0.7298	0.1033	0.859
			c'	0.9725	0.1522	0.844
			b'	0.8293	0.1495	0.820
			a'	0.6894	0.1178	0.829
	A-3	Stalks	c'	0.0996	0.0147	0.853
			b'	0.6523	0.0954	0.854
			a'	0.5625	0.0899	0.840
	A-1	Leaves	e'	5.4560	0.5161	0.905
			d'	4.5284	0.4521	0.900
			c'	4.0549	0.4266	0.895
			b'	1.9361	0.2163	0.888
			a'****	—	—	—
	A-2	Leaves	d'	5.1128	0.4923	0.904
			c'	3.9871	0.4216	0.894
			b'	1.7084	0.1765	0.897
			a'	0.1075	0.0112	0.896
	A-3	Leaves	c'	0.9063	0.0847	0.907
			b'	1.8905	0.1990	0.895
			a'	0.6173	0.0571	0.907

* The respective stocks were designated as A, B, E etc.
** No stalks were recognized for the sample harvested on 2008-11-30.
*** The bottom section (lower sectin of the stalk) was designated as a', and the data-columns were arranged in the order of the top to the bottom sections of the stalks
**** No leaves.

Table 2 Accumulation of iron (*Fe*)

Date of harvest	Samples	Tissues	Number of leaves	μg *Fe*/g dry weight
11/30/2008	A	Leaves	13	68.510
	B		9	112.811
	E		7	89.547
	F		12	108.641
	K		6	90.139

Date of harvest	Samples	Tissues	Sections	μg *Fe*/g dry weight
2/8/2009	A-1	Stalks	c'	160.745
			b'	37.978
			a'	46.860
	A-2	Stalks	b'	75.457
			a'	46.867
	A-3	Stalks	b'	—
			a'	39.955
	A-4	Stalks	b'	71.231
			a'	34.292
	A-1	Leaves	c'	51.538
			b'	23.863
			a'	25.953
	A-2	Leaves	b'	32.203
			a'	—
	A-3	Leaves	b'	28.393
			a'	103.685
	A-4	Leaves	b'	24.464
			a'	34.745

Date of harvest	Samples	Tissues	Sections	μg *Fe*/g dry weight
3/11/2009	A-1	Stalks	d'	70.878
			c'	22.505
			b'	23.047
			a'	31.866
	A-2	Stalks	d'	84.974
			c'	24.624
			b'	35.693
			a'	32.362
	A-3	Stalks	d'	209.689
			c'	25.375
			b'	32.374
			a'	143.516
	A-1	Leaves	d'	140.568
			c'	23.924
			b'	98.247
			a'	150.762
	A-2	Leaves	d'	57.540
			c'	100.862
			b'	70.425
			a'	142.623
	A-3	Leaves	d'	69.611
			c'	73.605
			b'	82.238
			a'	72.842

Date of harvest	Samples	Tissues	Sections	μg *Fe*/g dry weight
4/12/2009	A-1	Stalks	e'	42.770
			d'	89.777
			c'	84.749
			b'	25.465
			a'	32.293
	A-2	Stalks	d'	59.181
			c'	36.721
			b'	128.444
			a'	61.706
	A-3	Stalks	c'	352.925
			b'	68.398
			a'	72.041
	A-1	Leaves	e'	76.197
			d'	89.084
			c'	72.482
			b'	100.419
			a' ****	—
	A-2	Leaves	d'	63.597
			c'	81.489
			b'	138.449
			a'	380.418
	A-3	Leaves	c'	43.521
			b'	35.693
			a'	101.623

* The respective stocks were designated as A,B, E etc.
** No stalks were recognized for the sample harvested on 2008-11-30.
*** The bottom section (lower sectin of the stalk) was designated as a', and the data-columns were arranged in the order of the top to the bottom sections of the stalks.
**** No leaves.

iron concentration became greater in the samples harvested in March and April.

3. Accumulation of *Mg* (Table 3)

3-1. In the Hijiki plants harvested in November, the magnesium contents were several mg/g dry weight. These values correspond to 0.3 millimoles *Mg*/g dry weight of tissues.

3-2. In the leaves of the younger adult plants (Table 3, the samples harvested in February), the *Mg* levels were lower than those in the primary-stage leaves (the 1^{st} column of Table 3; samples harvested in November) or those in the later period of adult plants. In the leaves harvested in February, the magnesium contents were lower than those harvested in March and April. During the growth of the tissues, magnesium in the leaves seems to be three to four times more accumulated in the mature tissues in March and April.

3-3. In the stalks of February and March, the magnesium contents were similar to those of the November samples of primary leaf-stage plants. In April the magnesium concentration became higher to the level of the samples of the leaves harvested in March and April. The magnesium concentrations were rather similar between the samples along the stem, in comparison with the iron contents.

Discussion

The concentration of *Fe* in the primary-leaves was higher than in the younger leaves harvested in February, and *Mg* also showed higher accumulation in the primary leaves, as indicated in Fig. 2-a and 2-b. It is remarkable that the accumulation patterns of both elements, *Fe* and *Mg*, are not discrepant to each other. This may indicate that accumulation of both *Fe* and *Mg* occurred following the same time course, their concentrations approaching similar values. After the plants became matured, accumulation reached higher levels, although the accumulation in the stalks delayed compared to that in the leaves (Fig 2-c and 2-d).

A marine alga, *Sargassum* sp., harvested in Margarta Islands, Venesuela, accumulated *Fe* to an average concentration of 1,570 μg *Fe*/g dry algae (800 to 3,000 μg *Fe*/g dry algae)[11]. In Hijiki, the accumulation level was less than a few hundred μg/g dry weight. Thus, the accumulation level seems to differ greatly depending on the individual species. The accumulation mechanism in Hijiki is worthy of further investigation, as observed in *Ectocarpus*[12].

In the Ulva leaves, the *Mg* contents ascribable to chlorophylls are 0.13 mg/g dry weight of the tissues[13]. In the

Table 3 Accumulation of magnesuim (*Mg*)

Date of harvest	Samples	Tissues	Number of leaves	mg *Mg* /g dry weight
11/30/2008	A	Leaves	13	7.288
	B		9	8.615
	E		7	8.219
	F		12	7.375
	K		6	7.524

Date of harvest	Samples	Tissues	Sections	mg *Mg* /g dry weight
2/8/2009	A-1	Stalks	c'	4.155
			b'	7.065
			a'	8.191
	A-2		b'	5.619
			a'	6.159
	A-3		b'	—
			a'	5.866
	A-4		b'	8.539
			a'	7.359
	A-1	Leaves	c'	6.506
			b'	6.835
			a'	6.939
	A-2		b'	6.577
			a'	—
	A-3		b'	7.316
			a'	10.553
	A-4		b'	7.895
			a'	8.081

Date of harvest	Samples	Tissues	Sections	mg *Mg* /g dry weight
3/11/2009	A-1	Stalks	d'	6.847
			c'	8.877
			b'	10.388
			a'	8.570
	A-2		d'	9.252
			c'	9.829
			b'	9.532
			a'	8.042
	A-3		d'	9.239
			c'	9.099
			b'	9.914
			a'	9.637
	A-1	Leaves	d'	18.806
			c'	18.258
			b'	17.718
			a'	9.295
	A-2		d'	19.011
			c'	19.800
			b'	19.160
			a'	15.567
	A-3		d'	19.766
			c'	20.504
			b'	17.143
			a'	17.304

Date of harvest	Samples	Tissues	Sections	mg *Mg* /g dry weight
4/12/2009	A-1	Stalks	e'	21.594
			d'	22.243
			c'	21.876
			b'	20.015
			a'	21.380
	A-2		d'	29.868
			c'	30.031
			b'	20.934
			a'	23.041
	A-3		c'	21.823
			b'	25.465
			a'	26.862
	A-1	Leaves	e'	18.264
			d'	20.018
			c'	19.929
			b'	20.072
			a' ****	—
	A-2		d'	14.100
			c'	26.676
			b'	20.249
			a'	23.137
	A-3		c'	22.417
			b'	23.074
			a'	26.347

*, **, ***, ****: The same as described in the Table 2.

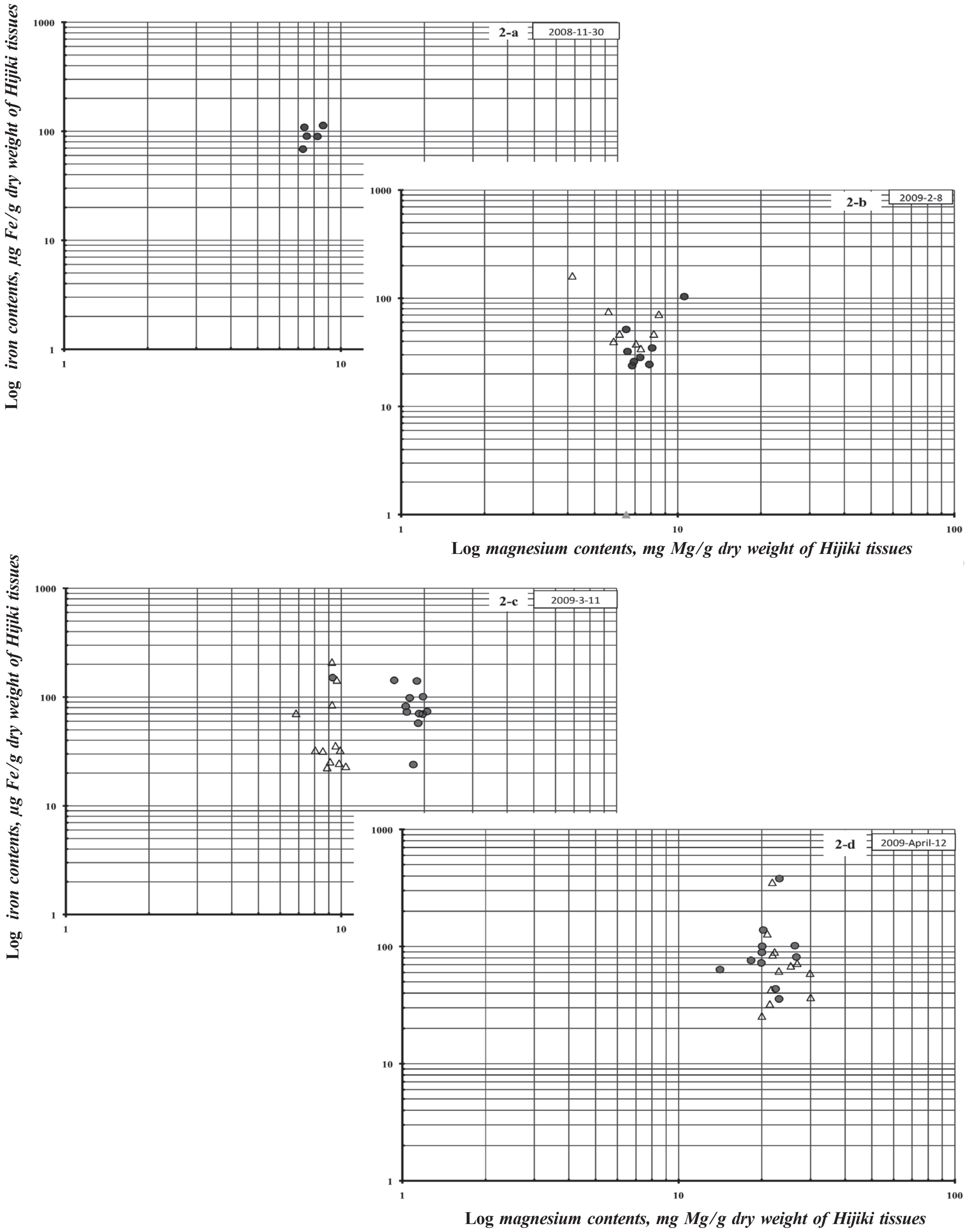

Fig. 2 Comparison between *Fe* and *Mg* accumulation patterns during the growth of Hijiki.
Concentrations of both elements in the stalks (△) and leaves (●) were plotted in the log scale.

terraneous plants, such as wheat cultivars, the *Mg* contents due to the chlorophyll contents were similar[14]. These suggest that *Mg* could be nearly ten times more accumulated in the tissues of Hijiki plants[3]. The greater concentration of magnesium in brown rice was contributed by the greater content of magnesium-phytate. In the case of Hijiki, some compounds like magnesium-phytate could contribute to the high contents of *Mg*.

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