Antioxidative activities of some dietary fibres determined by near infrared emission spectroscopy

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Introduction

We have been working on singlet oxygen, one of the active oxygen species, since 1988.[1] In this paper, we would like to describe the antioxidative activities of some dietary fibres determined by near infrared (NIR) emission spectroscopy as an application of singlet oxygen chemistry.

Singlet oxygen, superoxide, hydroxy radical and hydrogen peroxide are active oxygen species; in the medical field, ROOH, ROO, RO and HOX (X = Cl, Bl, or I) are also included in the term "active oxygen".

Active oxygen species are always generating in living bodies, either during the course of oxidative degradation of nutrients or of photosynthesis. Every living thing protects itself from the harmful effects of the active oxygen species using antioxidants, such as superoxide dismutase (SOD), β-carotene, catalase and vitamins C and E. Otherwise, they suffer cancer, inflammation or mutation and so on. As antioxidants cannot guard bodies perfectly, the result is ageing.
Antioxidants (AH_2) are thought to be radical scavengers or reducing agents.[1]

$$ROO^{\bullet} + AH_2 = ROOH + AH^{\bullet}$$

Dietary fibres are known to prevent cancer formation in the large intestine by their physical effects: (1) absorbing and holding water (faecal bulk increasing; promoting digestion and regulating stools);

Near Infrared Spectroscopy: Proceedings of the 10th International Conference

(2) absorbing toxic organic compounds (chromatographic action); (3) absorbing metal ions (positive ion-exchanging capability) and (4) gelling capability to result in (a) activation of digestive tracts; (b) increasing bulk of faeces; (c) accelerating faecal passage through digestive tracts; (d) decreasing internal pressure in the digestive tracts; (e) controlling digestion and/or absorption of diet constitution and (f) affecting intestinal bacteria.

Fucoidan, a marine dietary fibre, was also shown by us to prevent infection of some pathogenic virus by its immunological-like activity.[2]

Some dietary fibres have been known to be decomposed by active oxygen species:[3,4] They might have antioxidative properties. Do dietary fibres act as antioxidants? We intend to clarify that some dietary fibres have antioxidative activity, which may prevent cancer. We applied NIR emission spectroscopy to measure the antioxidative activity of some dietary fibres against singlet oxygen.

Materials and methods

Samples

Fucoidan (Sigma, St. Louis, USA), pectin (apple; Sigma), dextran sulfates Na 5000 and 500000 (Wako, Osaka, Japan), ι- and κ-carrageenans (Aldrich, Milwaukee, USA), alginic acid Na (Grade NB-S; Kimitsu Chem. Ind., Tokyo, Japan), NaN_3 (Wako) and vitamin C (Wako) were used as purchased. Fucoidan extracted from Okinawan mozuku *Cladosiphon okamuranus* was generously supplied by Miyako Kagaku Co. Ltd., Tokyo, Japan.

NIR emission spectroscopy

Singlet oxygen is an excited state and gives a dual emission, (1) red light from a dimer and (2) NIR light from a monomer, when deactivated. The red emission is, however, often obscured by emission from the other CL species. Therefore, it cannot be relied on for evidence of singlet oxygen. On the other hand, the NIR emission has no hindering emissions around them. Therefore, when 1270 nm emission is found, you can say safely, singlet oxygen must be present. We have constructed an NIR emission spectrophotometer (Figure 1).[5]

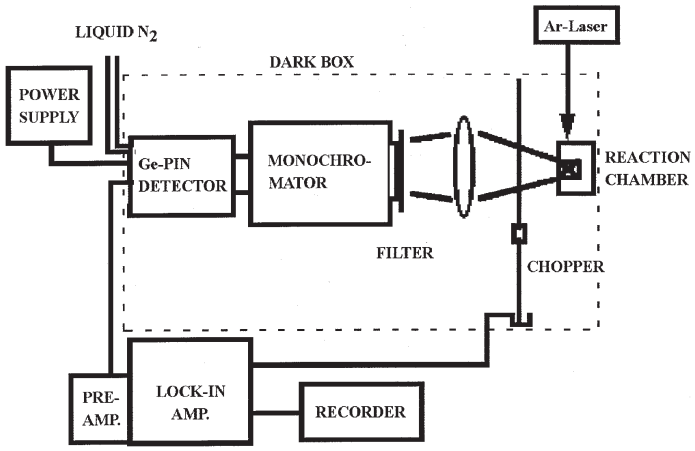

Figure 1. Diagram of the NIR emission spectrophotometer.

$$\text{Dye} + h\nu \rightarrow {}^1\text{Dye}^* \rightarrow \text{Dye}^*$$

$${}^3\text{Dye}^* + {}^3\text{O}_2 \rightarrow \text{Dye} + {}^1\text{O}_2$$

mol oxygen — singlet oxygen

Scheme 1.

There are many ways to make singlet oxygen: the photosensitised oxygenation method is the most convenient way among them. When a dye absorbs light, it is excited to give a singlet excited state, which transforms, sometimes, to give a triplet excited state. The triplet exited state transfers its excited energy to molecular oxygen (triplet ground state) to give excited singlet oxygen (Scheme 1).

Some diseases are known to result from singlet oxygen generation in a living thing as shown below:

(1) a cat eating abalones loses his ears;
(2) some inherited light-hypersensitivity;
(3) as well as a medical treatment, called photodynamic cancer therapy.

Emission spectra of singlet oxygen generated from an aqueous solution of Rose of Bengal under irradiation with a green laser (532 nm) were measured by the NIR emission spectrometer.

The quenching experiments were as follows: intensities of emission spectra were measured in the absence (I_0) and in the presence of the seaweed constituents (I) (Figure 2); ratios of I_0 / I were plotted against every concentration of the quenchers (Stern–Volmer plots) which gives a straight line (Figure 3). The slope of each line gives a $k_q\tau$ value, which gives a quenching constant k_q value (an antioxidative constant against singlet oxygen) when the τ value (half-life time of singlet oxygen in the solvent used) was given.[5]

A solution of a dye [Rose of Bengal (Aldrich); 5–10^{-4} mmol L^{-1}] and a quencher, antioxidant, was introduced into a flow-cell (1.2 mL min^{-1}) and continuously irradiated by a green laser (532 nm; CRGL-1100 (110 mW); CrownEO Co., Ltd.). The generated singlet oxygen was monitored by the

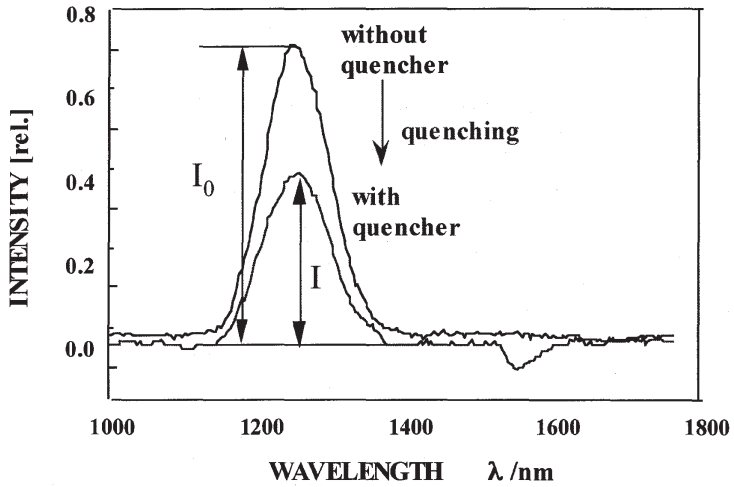

Figure 2. Quenching experiments of singlet oxygen using the NIR emission spectrometer.

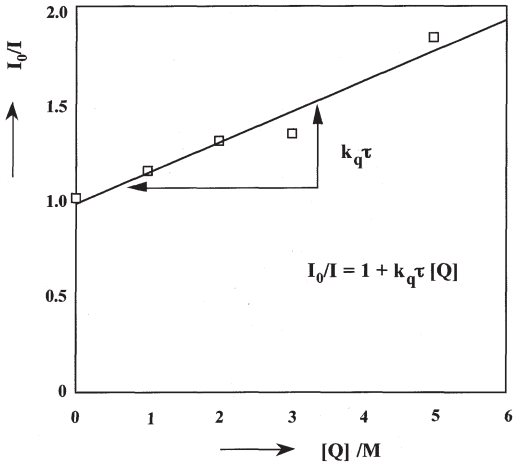

Figure 3. A Stern–Volmer plot for singlet oxygen quenching.

emission spectrophotometer and the spectra were observed. In the Batch type cell, dye could be bleached out and the quencher destroyed quickly.

Empirical calibrations

When measuring the emission intensities for dietary fibres, special consideration is needed to eliminate absorption effects: compensation was made at both 514 and 1100 nm.

Results and discussion

Table 1 shows antioxidative constants (quenching constants) of the dietary fibres. Quenching constants against superoxide, k_3, were also obtained using a method similar to that for singlet oxygen using chemiluminescence of a *Cypridina* luciferin analogue (CLA).[6]

The results obtained for dietary fibres show that fairly large k_q values were obtained from fucoidan, pectin, and Kombu extracts in g L^{-1}. They have also large k_3 values against superoxide oxidation as shown in Table 1.

Some dietary fibres, such as fucoidan (Figure 4), pectin (apple), and Kombu extracts showed antioxidative activity also for auto-oxidation (data not shown). They suppressed peroxidation of linoleic acid.

Several papers are found in the literature dedicated to oxidation of sugars by active oxygen species.[3,4] However, we believe, there are no papers on their quantitative antioxidative activity against active oxygen species.

Hyaluronic acid in connective tissues or synovia, a mucopolysaccharide, was reported to be depolymerised by active oxygen species to give inflammation of a joint or muscle. This reaction is suppressed by either catalase or SOD.[3]

Pectin and dextran are known to be depolymerised by ascorbic acid and Cu ion. Either an OH radical scavenger or quencher of singlet oxygen (DABCO) suppresses the reaction. Therefore, singlet oxygen can be concerned in the reaction as well as the OH radical and superoxide.[4]

Therefore, we examined the antioxidative activities of several representative "dietary fibres," such as fucoidan, pectin, carrageenans, alginic acid, dextran sulfate Na and Kombu extracts using the NIR emission spectrophotometer constructed in our laboratory and found them to quench singlet oxygen

Table 1. Antioxidative constants of dietary fibres.

Sample	MW	for $^1O_2$ $k_q/10^4$ (g L)$^{-1}$ s^{-1} ($k_q/10^{8\,M-1}$ s^{-1})		for O_2^- $k_q/10^4$ (g L)$^{-1}$ s^{-1} ($k_q/10^8$ M^{-1} s^{-1})	
		in EtOH-water (3 : 1) τ = 26.37μs		in water	
Vitamin C	176.13	1900[b] 8.94	34[b] (0.15)	400	(7.1)
NaN_3	65.01	401	(2.61)	—	
Kombu *Laminaria japonica*	—	10.34		4.4	
Fucoidan (from YT)	—	1.74		0.23	
Fucoidan (Sigma)	—	3.96 8.75[b]		[a]	
Alginic Acid Na	—	[a]		0	
ι-Carageenan	—	[a]		0.018	
κ-Carageenan	—	[a]		0.0074	
Pectin (apple)	20000–400000	1.16	(2.32–46.4)	0.32	(0.85–13)
Dextran Sulfate Na	5000	0.39	(0.19)	0.0041	(0.0041)
Dextran Sulfate Na	500000	0.07	(3.27)	0.056	(0.013)

[a]No data
[b]in water; $\tau = 2 \times 10^{-6}$ s

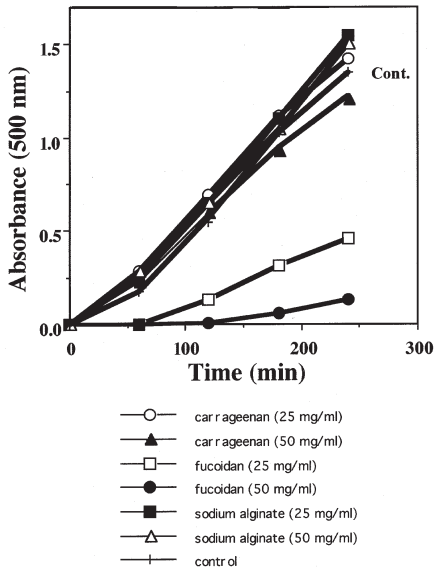

Figure 4. Antioxidative activity of some dietary fibres against autoxidation (POV).

($^1O_2$) as well as superoxide. Constituents of several representative seaweeds, such as wakame *Undaria pinnatifida*; hijikia *Hizikia fusifome* and kombu *Laminaria japonica*, were found to have fairly large reaction rates determined by quenching experiments of emission spectra in the NIR region (λ_{max}: 1270 nm) from $^1O_2$. The determined reaction rates are between 10^3–10^5 $(g\ L^{-1})^{-1}s^{-1}$; the larger ones are as large as that of ascorbic acid, 8.4×10^4 $(g\ L^{-1})^{-1}s^{-1}$. Some of these seaweed constituents also showed antioxidative activity against auto-oxidation and superoxide as well as their immunological enhancing activity.

These results suggest that dietary fibres, which are indigestible in the human body and can reach the large intestine without being hydrolysed, could scavenge either active oxygen species or toxic radical species to prevent carcinogen in the large intestine by their chemical, antioxidative activity.

Conclusions

1. Some "dietary fibres" were found to quench $^1O_2$ as well as O_2^- and to prevent autoxidation effectively. This suggests that they could work as antioxidative compounds in the alimentary canal (digestive organ).
2. Dietary fibres could prevent cancers of the colon by their chemical elimination of carcinogenic compounds as well as by known physical effects.

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