Antioxidative activity of animal and vegetable dietary fibres

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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 anti-oxidative activities of some animal and plant 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 the active oxygen species; in the medical field, ROOH, $ROO^{\bullet}$, NO and HOX (X = Cl, Br or I) are also included in the active oxygen.

Active oxygen species are always generating in living bodies, either in the course of oxidative degradation of nutrients or of photosynthesis. Every living thing protects themselves from the harmful effects of active oxygen species using antioxidants such as superoxide dismutase (SOD), β-carotene, catalase and vitamins C and E. Otherwise they suffer from cancer, inflammation or mutation, etc. As antioxidants cannot guard the body perfectly from them, then aging must result.

Antioxidants (AH_2) are supposed to be radical scavengers or reducing agents.[1]

$$ROO^{\bullet} + AH_2 = ROOH + AH^{\bullet} \quad (1)$$

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; (2) absorbing toxic organic compounds (chromatographic action); (3) absorbing metal ions (positive-ion exchanging capabilities); (4) gelling capability to result in (a) activation of digestive tracts, (b) increasing bulk of faeces, (c) accelerating faecal passage through the digest tract, (d) decreasing internal pressure in the digestive tract, (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 decompose by active oxygen species:[3] they could be chemically anti-oxidative. Do dietary fibres act as antioxidants? We intend to clarify that some dietary fibres have anti-oxidative activity and to prevent cancer chemically. We applied NIR

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emission spectroscopy to measure the anti-oxidative activity of some animal dietary fibres as well as plant fibres against singlet oxygen.

Materials and methods

Bovine serum albumin (BSA), egg albumin (Ovalbumin) and milk casein, purchased from Sigma Chemical Co. (St Louis, USA). Fucoidan (Sigma, St Louis, USA) pectin (apple: Sigma), dextran sulphates Na 5000 and 50,000 (Wako, Osaka, Japan) ι- and κ-carrageenans (Aldrich, Milwaukee, USA), alginic acid Na (grade NB-S, Kimitsu Chemical Industries, Tokyo, Japan), other sugars, NaN_3 (Wako), vitamin C (Wako) and triethylenediamine (DABCO, Wako) were used as purchased. Fucoidan extracted from Okinawan mozuku *Cladosphon okamuranus* was generously supplied by Miyako Kagaku Co. Ltd, Tokyo, Japan.

NIR emission spectroscopy

Emission spectra of singlet oxygen generated from an aqueous solution of Rose Bengal under irradiation of a green laser (532 nm) were measured by the NIR emission spectrometer that was developed in our laboratory (Figure 1)

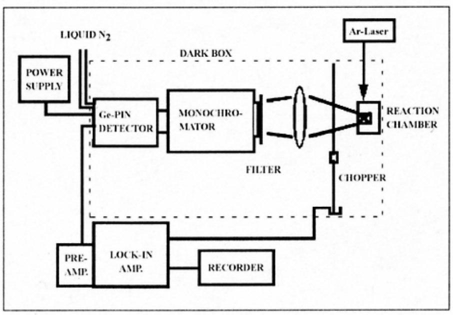

Figure 1. A diagram of NIR emission spectrophotometer

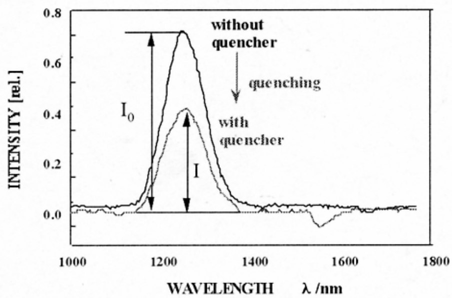

Figure 2. Quenching experiments by a quencher.

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). Every emission intensity (I_0/I) was calibrated by both the absorption percents at 532 and 1330 nm by each of the sample solutions. Ratios of the calibrated I_0/I were plotted against every concentration of the quenchers (Stern–Volmer plots), which gave 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 anti-oxidative constant against singlet oxygen) when the τ value (half-life time of singlet oxygen in the solvent used) was given.[4]

A solution of a dye [Rose Bengal, (Aldrich), 5×10^{-4} mmol L^{-1}] and a quencher, antioxidant, is 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 is monitored by the emission spectrophotometer and the spectra are observed. In the batch-type cell, dye could be bleached out and the quencher destroyed quickly.

Results and discussion

Table 1 shows antioxidative rate constants (quenching constants) of the dietary fibres. Quenching constants against superoxide k_3 were also obtained as for the singlet oxygen, using chemiluminescence of a *Cypridina* luciferin analogue (CLA).[5] The results obtained for dietry fibres show that fairly large k_q values were obtained from the animal and vegetable fibres such as sericin, BSA, casein, fucoidan, pectin and Kombu extracts in gL^{-1}. Some of them also have large k_3 values against superoxide oxide oxidation, as shown in Table 1.

Sericin, a byproduct protein produced from silkworms which has to be disposed of as an industrial waste, has been known to have anti-wrinkle properties and anti-oxidative activity to the auto-oxidation.[6] Some dietary fibres, such as fucoidan (Figure 4), pectin (apple) and Kombu extracts showed anti-oxidative activity 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] However, we believe there are only a few papers, published by us, on their quantitative anti-oxidative activity against active oxygen species.[3(c)] Hyaluronic acid in connective tissues or synovia, a mucopolysaccharide, was reported to be depolymerised by active oxygen species which inflamed joints or muscles. This reaction is suppressed by either catalase or superoxide dismutase (SOD).[3]Pectin and dextran are known to be depolymerised by ascorbic acid and Cu ion. Either an $^{\bullet}$OH radical scavenger or quencher of a singlet oxygen (DABCO) suppresses the reaction. So, singlet oxygen can be concerned in the reaction as well as OH radicals and superoxides.[3(c)].

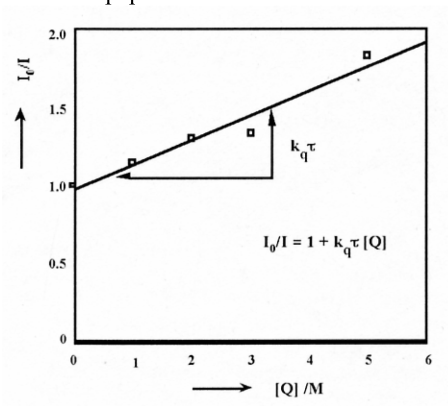

Figure 3. A Stern-Volmer plot for singlet oxygen quenching

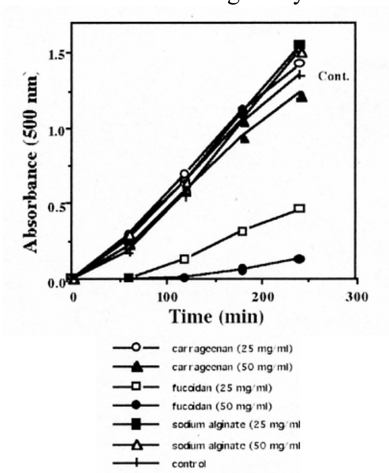

Figure 4. Anit-oxidative activity of some dietary fibres against auto-oxidation (POV).

Therefore, we examined anti-oxidative activities of several representative “dietary fibres” such as fucoidan, pectin, carrageenans, alginic acid, dextran sulphate Na and Kombu extracts using an NIR emission spectrophotometer constructed in our laboratory and found them to quench single oxygen ($^1O_2$) 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 high 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 high ones are as high as those of ascorbic acid 8.4 × 104 $(g\ L^{-1})^{-1}s^{-1}$. Some of these seaweed constituents also showed anti-oxidative 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 unhydrolysed, could scavenge either active oxygen species or toxic radical species by their chemical, anti-oxidative activity to prevent carcinogen in the large intestine.

Table 1. Anti-oxidative constants of dietary fibres in water

Sample	MW	for $^1O_2$ $k_q/10^4$ (g L)$^{-1}$ s^{-1} ($k_q/10^5$ M^{-1} s^{-1})	for O_2^- $k_q/10^4$ (g L)$^{-1}$ s^{-1} ($k_q/10^5$ M^{-1} s^{-1})
		In water; τ = 2.0 μs	
Sericin		35	
Bovine serum alb.	69k	250	
Ovalbumin	45k	97	
Casein	23.6k	868	
Pectin (apple)	20k–40k	470	0.32 0.85–1.3
Pectin (citrus)	20k–40k	479	0
Laminaran		202	0
Kombu *Laminaria japonica*		10.34	4.4
Fucoidan (Sigma)		8.75	—
Fucoidan (from YT)	—	1.74	0.23
Dextran sulphate Na	50,000	4.7	0.056 (0.013)
Dextran sulphate Na	5000	3.9	0.0041 (0.0041)
κ-Carageenan	—	0	0.0074
ι-Carageenan	—	0	0.018
Alginic acid Na		0	0
Starch		0	0
Dextrin		2.5	0.06
Maltose		0	0
Cellolobiose		0	0.12
Xylitol		0	0
D-glucose		6	0.09
D-fructose		4.35	0.24
D-mannose		0	0
Vitamin C	176.13	1.93 34	1900 (7.1)
NaN_3	65.01	0.04 2.6	0
DABCO	112.18	33.5	0

Conclusions

Some dietary fibres originating from animals such as the silkworm (Sericin) and others along with constituents of several representative seaweeds such as wakame *Undaria pinnatifida*, hijikia *Hizika fusifome* and kombu *Laminaria japonica*, were found to have fairly high reaction rates determined by quenching experiments of emission spectra in the near infrared region (λ_{max}, 1270 nm) from singlet oxygen ($^1O_2$). Emission spectra of singlet oxygen generated from an aqueous solution of Rose Bengal under irradiation with a green laser (532 nm) were measured by a near infrared emission spectrometer constructed in our laboratory and corrected by the absorption both at 532 and 1330 nm.

The quenching experiments were as follows: intensities of emission spectra were measured in the absence (I_0) and in the presence of seaweed constituents (I). Ratios of I_0/I were plotted against every concentration of the quenchers (Stern–Volmer plots) which gave a straight line. Correction was made for the absorption by the samples both at 532 and 1270 nm. The slope of each line gives a $k_q\tau$ value that gives a quenching constant k_q value (an anti-oxidative constant against singlet oxygen) when the τ value (half-life time of singlet oxygen in the solvent used) was given.

The determined reaction rates in water are between 10^4–10^6 (g L^{-1} s^{-1}) for the animal and the plant dietary fibres; the higher ones are as high as those of ascorbic acid, 1.9×10^4 (g L^{-1} s^{-1}). Most of these seaweed constituents also showed anti-oxidative activity against auto-oxidation and superoxide as well as their immunologically-enhancing properties.

These results suggest a possibility that dietary fibres that are supposed to prevent cancer of the large intestine by their physical properties may prevent the cancer, at least in part, by their chemical, anti-oxidative activity.

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

Acknowledgment

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