BBA 45782

REVERSIBLE INTERCONVERSION OF B850 AND B810 IN CHROMATOPHORES OF *CHROMATIUM* D AS INDUCED WITH DETERGENTS

YASUO SUZUKI, SIGEHIRO MORITA AND ATUSI TAKAMIYA

Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Hongo, Tokyo (Japan)

(Received August 12th, 1968)
(Revised manuscript received December 20th, 1968)

SUMMARY

The absorption changes of bacteriochlorophyll in the chromatophores of *Chromatium* D were investigated. Addition of non-ionic and cationic detergents and alcohols to the chromatophore suspension caused a conversion of B850 to B810. A similar result was obtained with treatments which reduce the water content of the chromatophores (*e.g.*, drying, treatment with higher concentrations of neutral salts or glycerol). On reducing the concentration of the detergents and alcohols in the chromatophore suspension by dialysis, the absorption spectrum of the chromatophores completely returned to its original state. A similar recovery of absorption spectrum was obtained by wetting the dried films of chromatophores or by diluting the salts or glycerol solution with water. No significant changes occurred in B890 during these interconversions.

These treatments also induced reversible changes in the fluorescence emission spectrum of bacteriochlorophyll and the action spectrum of photo-induced oxidation of *c*-type cytochrome in the chromatophore.

INTRODUCTION

It is known that in most photosynthetic bacteria, the absorption spectrum of bacteriochlorophyll in intact cells or chromatophores shows three distinct peaks in the near infrared region, at 800–810 mμ, 850 mμ and 870–890 mμ (ref. 1). The corresponding forms or states of bacteriochlorophyll *in vivo* are designated B800, B850 and B890 after DUYSENS[2]. In contrast, purified bacteriochlorophyll in organic solvents shows only one absorption maximum at about 770 mμ (for instance, at 772 mμ in ether)[3]. The ratios of the heights of the three absorption peaks differ from species to species of the bacteria[1] and also according to the culture conditions of the cells, such as light intensity[4,8–10] and composition of nutritional medium[4–7]. The coloured carotenoid-less mutant of *Rhodopseudomonas spheroides* shows only two peaks of the absorption spectrum, namely, 810 mμ and 880 mμ; the 850-mμ peak is not present[11]. On the

Abbreviations: Triton X-100, alkylphenoxy polyethoxy ethanol; TL-10, polyoxyethylene sorbitan monolaurate (Nikko Chemical Co.); TO-10, polyoxyethylene sorbitan monooleate (Nikko Chemical Co.); Sanizol-C, alkylated dimethyl benzene ammonium chloride (Kaoh Soap Co.).

other hand, the absorption spectrum of the chromatophores of *Chromatium* D grown in a medium containing diphenylamine showed an infrared spectrum similar to that of the normal cells, in spite of the low content of coloured carotenoids in the cells grown under these conditions[12].

A pattern of the near infrared absorption spectrum of bacteriochlorophyll similar to those of the intact cells has been obtained with solutions of pure bacteriochlorophyll under conditions in which aggregates of bacteriochlorophyll are formed. Colloidal suspensions of bacteriochlorophyll[13], thin layers of bacteriochlorophyll dried on a glass surface[14], and microcrystals of bacteriochlorophyll were measured[15]. In some cases, an exact copy of the absorption bands of bacteriochlorophyll *in vivo* was obtained.

These differences in the absorption spectrum of bacteriochlorophyll are important, since they indicate different states of the pigment in the bacterial cell. In the present study the effects of some detergents and alcohols and some treatments which reduce the water content of chromatophores were found to cause reversible conversion of B850 to B810 in the chromatophores of *Chromatium* D. These treatments were also found to indicate reversible changes in the fluorescence emission spectrum which is a means of identifying the states of bacteriochlorophyll *in vivo*. The effects of the various treatments on the excitation energy transfer in the chromatophore photosynthetic pigments were tested by comparing the changes in the excitation spectrum of fluorescence and in the action spectrum of light-induced oxidation of *c*-type cytochrome in the chromatophores.

MATERIAL AND METHODS

Chromatium D was grown anaerobically according to the method of BOSE[16]. After 2–3 days of growth in a basal medium, the cells were collected by centrifugation, resuspended and grown for an additional 2–3 days in the same medium (starved cells). The starvation reduced the content of the sulfur granules in the cells and facilitated the preparation of chromatophores. The cells were harvested, washed once while centrifuging and suspended in 0.1 M phosphate buffer (pH 7.8), containing 0.25 M sucrose and 1 % NaCl. The cells in the suspension were subjected to disruption in an ice-cooled French pressure cell at 1200 kg/cm^2. The chromatophore fraction was obtained by fractional centrifugation, with the material sedimenting between 12000 $\times$ g for 20 min and 80000 $\times$ g for 1 h being collected. The chromatophore fraction thus obtained was designated "original chromatophore" and used for further experiments.

For the measurement of the absorption and difference spectra of the chromatophores, a Shimadzu Multipurpose Spectrophotometer (MPS 50, Shimadzu Co.) was used. All measurements were carried out at room temperature.

Fluorescence emission spectra of the chromatophores were measured according to the method of MURATA[17], slightly modified as follows. The excitation light with a band-width of 20 mμ at 590 mμ was supplied by a Bausch and Lomb grating monochromator. The fluorescence was analysed using another Bausch and Lomb grating monochromator. The intensity of fluorescence was measured with the use of a dry-ice-cooled infrared-sensitive photomultiplier (HTV 7102). The signal was amplified and recorded on a strip chart servo-recorder. In order to exclude the stray light, a visible light absorbing filter (Corning 9780) was used. Another cut-off filter, VR-69 (Toshiba

Electronic Co. Ltd.), was inserted between the sample and the detecting monochromator. The amounts of the sample used in the measurement were adjusted to give an absorption of less than 10 % of the incident light at the wavelength of maximum absorption (810 mμ).

The measurement of the action spectrum for the photo-induced oxidation of the *c*-type cytochrome (cyt-555) in the chromatophores was performed in an Aminco Chance Dual Wavelength Spectrophotometer. The actinic light was supplied by a Bausch and Lomb grating monochromator (band-width, 10 mμ). A cut-off filter, VR-69, was inserted between the actinic light and the sample. An infrared-absorbing filter (Corning 9780) was inserted between the measuring sample and the photomultiplier. The measuring wavelengths were set at 422 mμ and 460 mμ to correspond to the peak of maximum deflection (light *minus* dark) and to the isosbestic point in the Soret region of cyt-555. The concentration of bacteriochlorophyll was determined by the method of WEIGL[18], assuming a value of $0.23 \cdot 10^5$ for $\varepsilon_{575 m\mu}$ in ether. The concentration of chromatophores was adjusted to give an absorbance of 0.5 at 810 mμ.

RESULTS

Fig. 1 presents the absorption spectra of *Chromatium* chromatophores as influenced by the presence of Triton X-100 in the medium. Curve A in Fig. 1 shows a typical absorption spectrum of the state of bacteriochlorophyll *in vivo*. On addition of Triton X-100 to the medium, a decrease occurred in the absorption at 850 mμ accompanied by a concomitant increase at 810 mμ (Curves B–D). The change was observed more clearly in the difference spectra of the chromatophores (detergent-treated *minus* untreated) shown in Fig. 2. The change was fairly rapid, being accomplished within 1 min after addition of the detergent. No further change in shape of absorption spectrum nor in heights of the absorption peaks was detected on incubation for a few hours. Therefore in the following experiments, the absorption

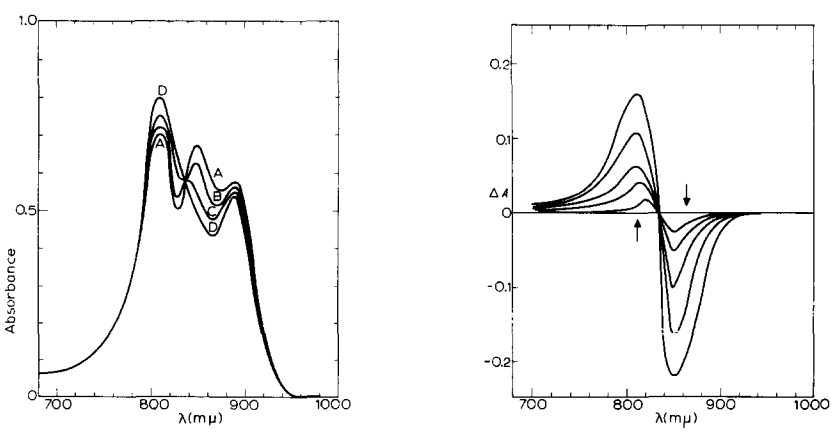

Fig. 1. Absorption spectra of *Chromatium* chromatophores. Curve A, "original chromatophores"; B, in presence of 1 mM Triton X-100; C, 5 mM Triton X-100; D, 10 mM Triton X-100.

Fig. 2. Difference spectra of *Chromatium* chromatophores (Triton X-100-treated *minus* untreated). Concentrations of Triton X-100 are 1–10 mM. Arrows indicate increasing concentrations of Triton X-100. The isosbestic point is at 837 mμ.

spectra were measured after 5–10 min of incubation in the presence of the detergents used.

The spectral change goes through an isosbestic point at 837 mμ (Figs. 1 and 2), and there is a linear proportionality between the magnitudes of increase at 810 mμ and the decrease at 850 mμ as long as the concentration of the detergent in the

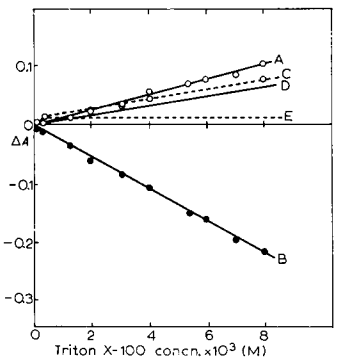

Fig. 3. Dependence of the change of bacteriochlorophyll forms on the concentration of Triton X-100. A (○—○), increase of B810; B (●—●), decrease of B850; C (○----○), estimated increase of B820; D (——), contribution of B810 to the absorbance at 820 mμ, was estimated to be 70% that of the maximum absorbance of B810 at 810 mμ; E (----), C *minus* D, namely, true increase of B820.

medium is low (Fig. 3). These facts indicate that the change observed in absorption spectrum reflects a conversion of one form of bacteriochlorophyll to another, *i.e.*, B850 to B810. In Figs. 1 and 2, it was noticed that at low concentrations of Triton X-100 (1 mM), a small band appeared at about 820 mμ. The intensity of the band at 810 mμ increased at higher concentrations of Triton X-100. This fact was also observed in Fig. 3. BRIL[19] observed the increase at 820 mμ when he treated *Chromatium* chromatophores with Triton X-100. He suggested that this 820-mμ band was derived from B850. As long as the concentration of Triton X-100 is low, our results favour his interpretation. At higher concentrations of Triton X-100, however, there was an increase at 810 mμ but no further increase at 820 mμ, as may be seen in Fig. 3. The 890-mμ band (B890) did not change during the transformation of B850 to B810, as shown in Fig. 2. On increasing the concentrations of the detergent above 10 mM, there was a gradual decrease in height of the absorption peaks at 890 mμ and 810 mμ. These changes were found to be irreversible and were inferred to be due to the photooxidation or some other degradation of bacteriochlorophyll in the chromatophores as reported previously, by other investigators, *e.g.*, IZAWA, *et al.*[20], BRIL[19] and GOEDHEER[21].

The reversibility of the detergent-induced conversion of B850 to B810 was tested by dialyzing the detergent-treated chromatophores in a cellophane tube against a large volume of the buffer solution. The initial shape of the absorption spectrum was completely recovered. The reversal of the detergent-induced change was also obtained by passing the detergent-treated sample through a Sephadex column (Sephadex G-100). The conversion of B850 to B810 in the presence of Triton X-100 and its reversal on reduction of the detergent could be repeated several times without any detectable loss of bacteriochlorophyll in the chromatophores.

The effects of other detergents on the absorption spectrum of the chromatophores

were also examined, and the results obtained are summarized in Table I. Nonionic detergents such as TL-10, TO-10 and digitonin, have effects similar to Triton X-100. A cationic detergent, Sanizol-C, exhibited similar effects at lower concentrations (0.1–1 mM). All these changes were also found to be reversible in the sense that the original absorption spectrum of the chromatophores was restored on removal of the detergents in the manner described for Triton X-100. Above the range of concentration indicated for each detergent in the table, there occurred an irreversible destruction of bacteriochlorophyll in the chromatophores just as was the case in the experiments with Triton X-100. Anionic detergents, such as sodium dodecyl sulfate, deoxycholate, sodium dodecyl benzene sulfonate and sodium cholate, did not cause such reversible conversion of B850 to B810. At higher concentrations of these substances there occurred an irreversible bleaching of bacteriochlorophyll in the chromatophores. This bleaching was most marked with respect to B890. Such differences in behaviour of the anionic detergents and the cationic and nonionic detergents have to be elucidated.

TABLE I

INFLUENCE OF DETERGENTS ON BACTERIOCHLOROPHYLL FORMS *in vivo*

Values indicate minimum and maximum concentrations of detergents which cause reversible conversion between B850 and B810.

Reagents	*Concn. (mM)*	
	Min.	*Max.*
Triton X-100	1.6	16
Digitonin	10	100
TL-10	10	100
TO-10	10	100
Sanizol-C	0.05	0.15
Sodium dodecyl benzene sulfonate	—	
Cholate	—	
Deoxycholate	—	
Sodium dodecyl sulfate	—	

TABLE II

INFLUENCE OF ALCOHOLS ON BACTERIOCHLOROPHYLL FORMS *in vivo*

Values indicate minimum and maximum concentrations of alcohols which cause reversible conversion between B850 and B810.

Reagents	*Concn. (mM)*	
	Min.	*Max.*
Ethyl alcohol	5000	
Propyl alcohol	1500	3000
n-Butyl alcohol	100	1000
n-Amyl alcohol	100	1000
Isoamyl alcohol	100	1000
n-Hexyl alcohol	10	100
n-Heptyl alcohol	1	10

Methyl cellosolve, ethyl cellosolve and polyethylene glycol exerted the same effect as Triton X-100 and other nonionic detergents.

The effects of alcohols on the chromatophores were also examined and similar results were obtained as with Triton X-100. These results were summarized in Table II. As will be seen from the table, alcohols having a longer chain of carbon atoms were more effective in causing the reversible change of B850 to B810.

The effects of sodium salts of fatty acids were also tested. This class of substances was ineffective in causing any spectral change of bacteriochlorophyll *in vivo*, nor was any sign of extraction of bacteriochlorophyll from the chromatophores detected.

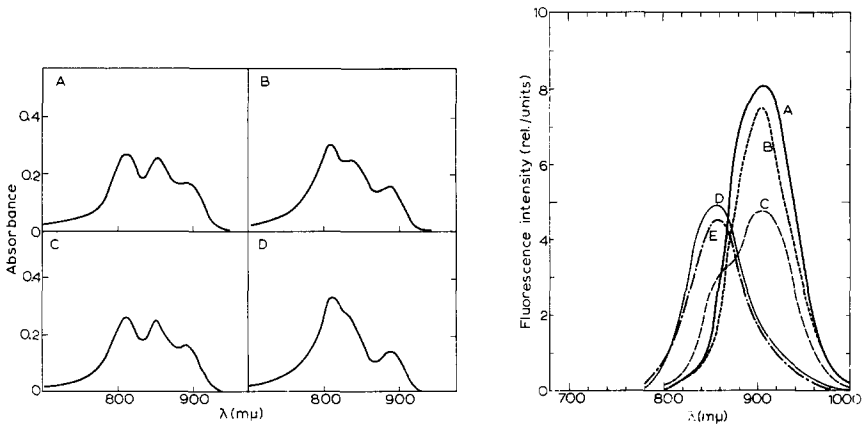

Fig. 4. Absorption spectra of *Chromatium* chromatophores. (A) "Original chromatophores"; (B) chromatophores spread on a glass plate; (C) B after gradual drying in air; (D) C sprayed with water.

Fig. 5. Fluorescence emission spectra of *Chromatium* chromatophores. A(——), "original chromatophores"; B(– – – –), 0.1 mM Trition X-100; C(– – –), 1 mM Triton X-100; D(——), 5 mM Triton X-100; E(— · —), 10 mM Triton X-100.

On drying and wetting the films of chromatophores spread on the surface of slide glass, there occurred reversible changes in the absorption spectrum similar to those corresponding to the interconversion between B850 and B810 as observed on detergent treatment of the aqueous suspension of chromatophores (Fig. 4). CLAYTON[22] also has observed a reduction of the absorption band at 859 mμ (B850) in dry films of *Chromatium* chromatophores. Other reagents and treatments which might cause "dehydration" of the chromatophores were also tested to examine the effects of "dehydration" on the absorption spectrum. The chromatophores were suspended in a medium containing 80% glycerol. The absorption spectrum showed a markedly higher content of B810 than of B850. A similar finding was obtained with the pellet of chromatophores obtained by sedimenting the chromatophores with ammonium sulfate. In both cases the original absorption spectrum of the chromatophores was completely restored when the media were diluted by addition of water. The term "dehydrating" does not necessarily mean a complete dehydration of chromatophore substance under these experimental conditions but a reduction of the water content of chromatophores which might cause certain structural changes of chromatophores.

For detecting the effects of the detergents (and other treatments) on the transfer

of excitation energy in bacteriochlorophyll *in vivo*, the influence of the reagents on the fluorescence of bacteriochlorophyll in the chromatophores was examined. Fig. 5 shows the emission spectrum of the chromatophores as affected by the presence of varied concentrations of Triton X-100. At a Triton X-100 concentration as low as 0.1 mM (Fig. 5, Curve B), which caused no detectable change in the absorption spectrum, there was a detectable depression of fluorescence, although there was no change in position of the emission band (910 mμ). On increasing the detergent concentration up to 1–10 mM (Fig. 5, Curves C–E), the emission band at 910 mμ (F910) disappeared to be replaced by a new one at 860 mμ, concomitant with a marked decrease in total yield of fluorescence. This is interpreted as a result of a progressive deterioration of energy transfer from B850 to B890 caused by the action of the detergent. The fluorescence band at 860 mμ is assumed to be emitted from B820 or from B810. Considering the position of the absorption maximum, the 860-mμ band must originate from B820 rather than from B810. The possibility of the 860-mμ band originating from B850 had been excluded by BRIL[19]. Our results agree with his. The complete disappearance of the 910-mμ band from the emission spectrum under these conditions must be a result of quenching of the fluorescence of B890, since on excitation with the actinic light at 590 mμ, all three forms of bacteriochlorophyll, including B890, must have been excited by the illumination. The observed decrease in fluorescence yield might have been caused by the lowering of efficiency of the transfer of the energy in bacteriochlorophyll *in vivo*.

These effects of Triton X-100 on fluorescence properties of the chromatophores were found to be completely reversed on removal of the detergent as was described for the changes in the absorption spectrum.

Similar reversible changes in the emission spectrum were encountered on treatment of the chromatophores with alcohols and Sanizol-C, as well as on treatment with 80 % glycerol at the concentration ranges within which the changes in absorption spectrum were reversible.

Fig. 6 shows the action spectra for the photo-induced oxidation of cytochrome

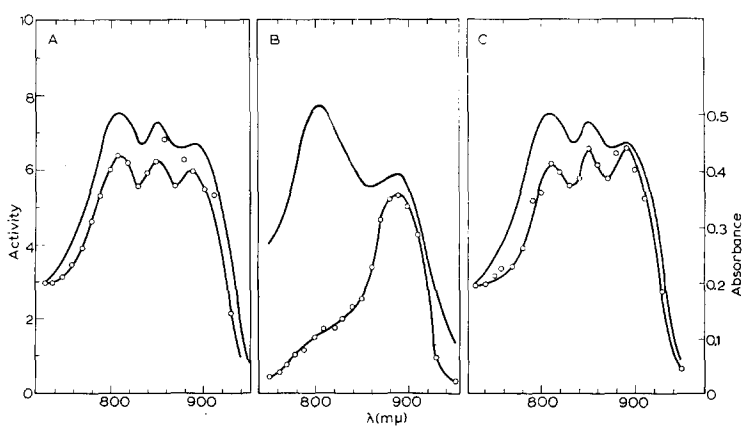

Fig. 6. Action spectra (O—O) for light-induced oxidation of cytochrome *c*-555 in the chromatophores of *Chromatium*. (A) "Original chromatophores"; (B) treated with *n*-heptyl alcohol (10 mM); (C) as B, *n*-heptyl alcohol removed by dialysis. ——, absorption spectra of chromatophores. Activity was expressed by reciprocal of light intensity to induce the absorbance change (ΔA = 0.00434).

(cyt-555) in the presence and absence of added *n*-heptyl alcohol. The action spectrum with the original, untreated chromatophores (Fig. 6A) shows that the light energy absorbed by B810 and B850 was transferred with a fairly good efficiency to B890, resulting in the photo-induced oxidation of cytochrome. The efficiencies of the energy transfers from B810 and from B850 to B890 are lowered by the treatment with *n*-heptyl alcohol, as will be seen from the action spectrum shown in Fig. 6B. On removal of the alcohol from the reaction mixture, the original activity for photo-induced oxidation of cytochrome and, consequently, high efficiency of energy transfer were completely recovered (Fig. 6C). Similar reversible effects were also obtained with Triton X-100 or with 80% glycerol.

It is concluded that treatment with some detergents and alcohols induced reversible changes in states of bacteriochlorophyll in the chromatophores, which may be denoted as a conversion of B850 to B810 (and to B820). These treatments also affect the energy transfer in the three forms *in vivo*, causing marked changes in fluorescence properties of the chromatophores as well as changes in the action spectrum for the light-induced oxidation of cytochrome in the chromatophores.

DISCUSSION

During the present study it was found that there are three categories of treatments which cause quite similar reversible changes in the absorption spectrum of bacteriochlorophyll in *Chromatium* chromatophores; (a) treatments with nonionic and cationic detergents (*e.g.*, Triton X-100, Sanizol-C), (b) treatments with alcohols, and (c) dehydration, including drying and treatments with inorganic salts or glycerol at higher concentrations. From the marked similarity in the nature of the changes observed, it may be inferred that all these apparently different treatments induce a common change in the state of bacteriochlorophyll in the chromatophores, designated as an interconversion from B850 to B810.

The efficiency of energy transfer in the photosynthetic pigments as estimated by the action spectrum for the photo-induced oxidation of *c*-type cytochrome in the chromatophores, was also found to be affected in a similar way by all three types groups of treatment in question. In these cases the decrease in the level of B850 as a result of its conversion to B810 seemed to be a principal cause of the diminished efficiency of energy transfer from B810 to B890, which is supposed to take place *via* B850. BRIL[19] has also discovered (by means of fluorescence excitation spectrum) a depression of energy transfer in the presence of a low concentration of Triton X-100. He also observed the reversibility of the change in the emission band of bacteriochlorophyll on treatment of *Chromatium* chromatophores with sodium deoxycholate.

Another feature concerning the state of bateriochlorophyll was furnished by the study of fluorescence of bacteriochlorophyll in the chromatophores. The treatments with the detergents and with alcohols were shown to affect the fluorescence in a similar and reversible way. However, the fluorescence, as measured by the yield in F910, was found to be more sensitive towards the detergent treatments in that there was a significant quenching of fluorescence at 910 mμ even at low concentrations of the detergents at which there was no detectable change in the absorption spectrum. The observed quenching of F910 cannot be solely ascribed to a blocking of the energy transfer from B850 and B810 to B890. There was no emission of F910 even when the chromatophores were illuminated with 590-mμ light which would

excite all the forms of bacteriochlorophyll *in vivo*, including B890 which emits F910. A mechanism must be operating which quenches F910 without changing the absorption characteristics of B890.

A concept of bacteriochlorophyll–bacteriochlorophyll interaction[14,15] is based on the findings of spectral shifts of bacteriochlorophyll observed under conditions of aggregation of bacteriochlorophyll. Along this line of reasoning, the observed conversion of bacteriochlorophyll on treatment of the chromatophores with detergents and with alcohols may be explained as follows. The detergents and alcohols combine with, or are adsorbed to, the lipid layer of the chromatophore membrane to which the molecules of bacteriochlorophyll are attached by their phytol tail, and cause changes in the structure or conformation of the chromatophores. These changes may in turn induce changes in distance or configuration of closely spaced bacteriochlorophyll molecules. Such changes may readily result in changes in electronic interaction between bacteriochlorophyll molecules.

BRIL[19] has reported that detergents act differently on various species of photosynthetic bacteria. We have also observed similar diversity in detergent action as noticed by BRIL, in *R. spheroides*, *R. rubrum* and *R. palustris*. Such differences might be caused by the higher resistance of the chromatophore structure of these organisms to detergents.

ACKNOWLEDGEMENTS

The authors wish to acknowledge with thanks aid they received from the Yamamoto-Nori Co. The present work was supported by a grant from the Ministry of Education.

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