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Resonance Raman Spectroscopies of Hemeprotein and Its Model Compound
Part 1 Purifications of Chlorophyll a and Bacteriochlorophyll, and These Partial Properties

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Introduction

This experiment is one part of the series of spectroscopic determination of organic material. Moreover, this part is one part of the studies related to hemeprotein and its model compound. In this case, chlorophyll a and bacteriochlorophyll were selected as the organic materials. Because they were related closely to very important photosynthesis for heterotroph. This chlorophyll a is so-called collected name. Properly speaking, chlorophyll a must be separated to chlorophyll a_1, a_2, a_3, ..., at least if it is classified or distingushed in detail. Before the specific property of each chlorophyll a was not determined, so such a name was used in this case. Of course, the determination of each specific property is carried on to publish the experimental results. The strict determination may be required more.

To purifications of chlorophyll a and bacteriochlophyll these used solvents and reagents were at the highest grade and purified more.

Chlorophyll a was isolated from fresh spinach with a partial modified method of Strain et al. very carefully.

Laboratory of Biology

"Spectroscopic Determination of Organic Material, Part 2: Resonance Raman Spectroscopy of Hemeprotein and Its Model Compound; Some Physicochemical Properties of Chlorophyll a and Bacteriochlorophyll" was the title of this experiment that the author expected. However, in relation of Specific Fund of Tokyo Kasei University, the title of this paper had to be described as the above description.

The author took care of the property and the purity when columns or carriers were used very carefully.

Bacteriochlorophyll was isolated and purified in the process of *Rhodospirillum rubrum* culture with a partial modified method of Cohen-Bagire very carefully. Moreoever, the buffer solution of HEPES was used in the stead of Tris·HCl buffer. And its effect was detected.

These various properties of purified chlorophyll a and bacteriochlorophyll were determined with spectroscopies of ultraviolet and infrared (usual method, and H⇌D film method: the author[1]) and resonance Raman (usual method using some improved cells).

This is the one part of experiment that was published at Conference[13] (368) of Japan Agricultural Chemical Society at Kyoto University.

The specific (fixed specially) fund of experiment for this Resonance Raman Sepctroscopy of Hemeprotein and Its model Compound was supported by Tokyo Kasei University.

Experimental and Results

The performed procedures of isolation, purification, and determinations of various properties related to chlorophyll a and bacteriochlorophyll should be described in detail. However, as the limitation of page in number is very strict at our university, the simplified description of this section (Experimental and Results; ex. detail procedure) or others had to be performed like this paper (as part 1). The rest detail procedures and the parts which could not be reported might be published on the next paper (as part 2). The contents of these two report papers

were published at the conference of Japan Agricultural Chemical Society. According to the above described publication, the main points including the results are written as following.

Now, many various reagents, solvents and materials (ex. adsorbents) were purified completely carefully.

Isolation and purification procedure of chlorophyll a:

This chlorophyll a was isolated and purified with the method of Strain et al. which was partially improved by the author.

Isolation and purification procedure of bacteriochlorophyll:

The used strain (*Rhodospirillum rubrum*) was cultured with the Cohen-Bagire's method which was partially improved by the author. And the method which was partially improved by the author was applied to this isolation and purification of the bacteriochlorophyll. The effect of usage of HEPES buffer in the stead of Tris·HCl buffer was examined.

The determined results: Chlorophyll a → Chl, Deuterated Chlorophyll a → D-Chl, Bacteriochlorophyll → Bact. Chl.

(a) absorption properties for ultraviolet range; Chl, D-Chl, Reduced Chl, Bact. Chl.; Fig. 1, 2, 3.

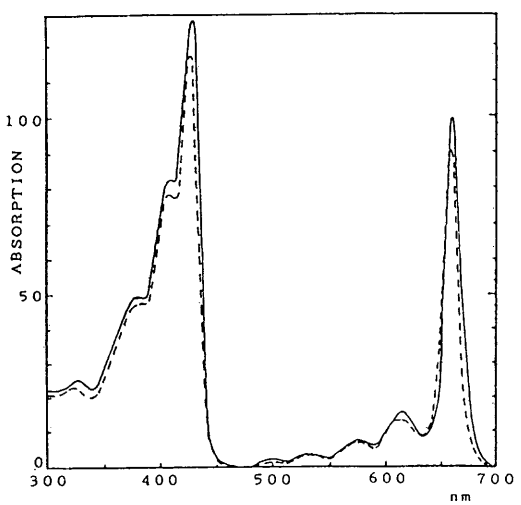

Fig. 1 Ultraviolet absorption spectra of chlorophyll (hydrated —) and chlorophyll (deuterated ---)

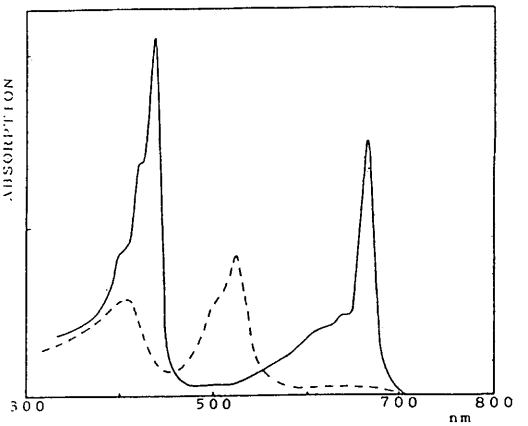

Fig. 2 Ultraviolet absorption spectra of chlorophyll (pyridine —) and chlorophyll (including ascorbic acid: reduced state ---)

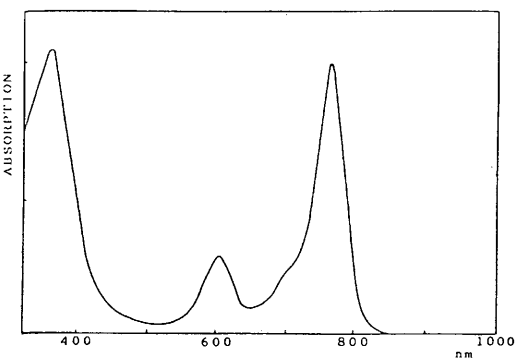

Fig. 3 Ultraviolet absorption spectrum of bacteriochlorophyll

(b) spectroscopic determination in infrared range; Chl, D-Chl, Bact. Chl.; Fig. 4, 5, 6, 7.

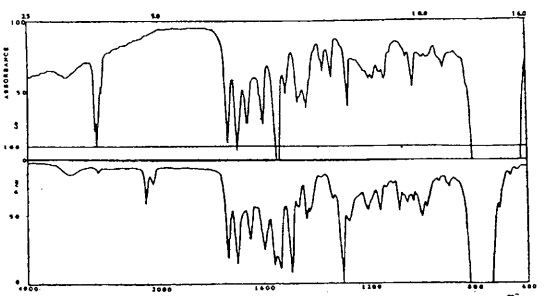

Fig. 4 Infrared absorption spectra of chlorophyll (upper: hydrated, lower: deuterated)

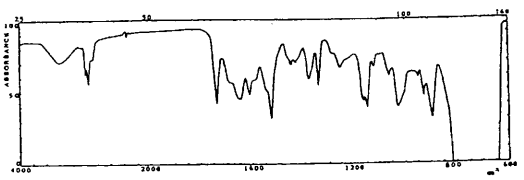

Fig. 5 Infrared absorption spectrum of bacteriochlorophyll

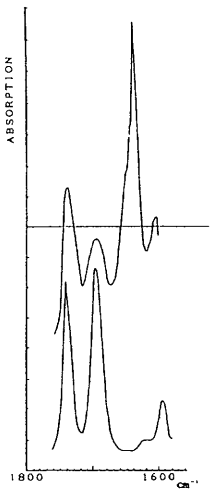

Fig. 6 Infrared absorption spectra of chlorophylls (upper: carbon tetrachloride, lower: tetrahydrofran

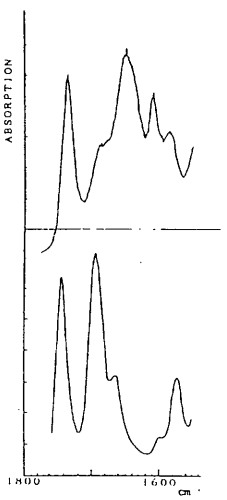

Fig. 7 Infrared absorption spectra of bacteriochlorophylls (upper: carbon tetrachloride, lower: tetrahydrofran)

(c) absorption spectra in far-infrared range; Chl.; Fig. 8.

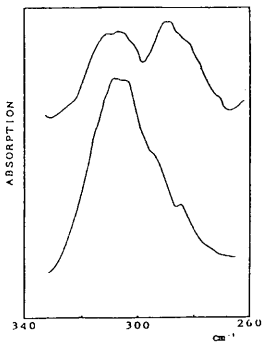

Fig. 8 Far-infrared absorption spectra of chlorophylls (upper: nujol, lowr: benzene)

(d) resonance Raman spectra; Chl.; Fig. 9, 10, 11, 12.

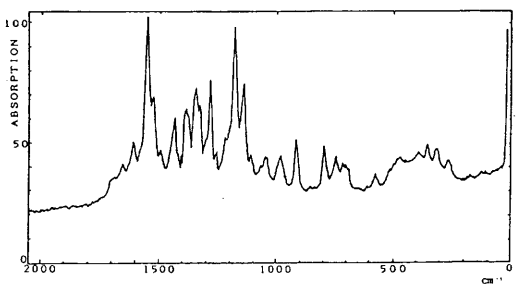

Fig. 9 Resonance Raman spectrum of chlorophyll (hexane)

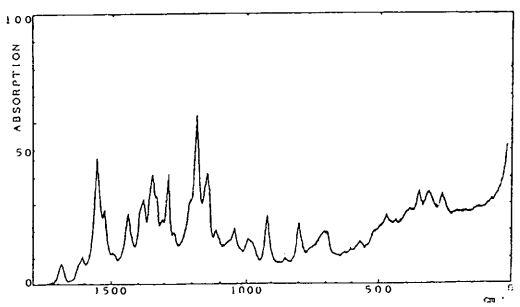

Fig. 10 Resonance Raman spectrum of chlorophyll (acetone)

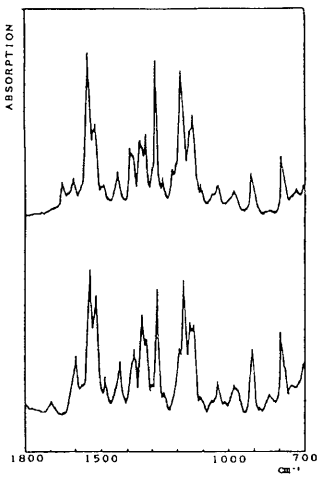

Fig. 11 Resonance Raman spectra of chlorophylls (upper: carbon tetrachloride, lower: ethyl ether)

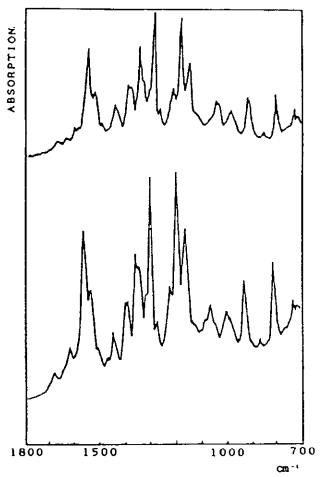

Fig. 12 Resonance Raman spectra of chlorophylls (upper: methyl alcohol, lower: ethyl alcohol)

In the case of Bact. Chl., a little fluorescence was showed, but the improved determination was performed. The one part of determination succeeded, the one part had to be improved.

Then, from the above described reason, the numerical results (lists) of the wave lengths or frequencies of absorption peaks or bands which were determined and corrected for each determination were not unexpectively able to be described on this paper. So, they may be published on the next paper. Of course, all absorption peaks or bands were tried to assign. On the other hand, they were published at the above-described conference.

Chlorophyll a

Procedure

1 kg spinach leaves free of midribs

↓ dropped into boiling water (ca. 4 liters), after 1.5 mins, were cooled quickly with cold water, decanted through a sieve, were rinsed once with cold water, squeezed by hand, between paper and cloth towels.

ca. 180 g wet weight of leaves

↓ placed in methanol 1200 ml plus petroleum ether 600 ml (bp 30°C), agitated for 30 mins.

deep green extract

↓ reextracted with methanol and petroleum ether, washed with petroleum ether 300 ml. The pigments transferred to petroleum ether. Each methanol plus petroleum ether extract was diluted 1 liter of saturated salt (NaCl) solution, thereby, transferring most of pigment to petroleum ether layer, all the petroleum ether layers were combined and evaporated to dryness at 35°C.

ca. 3.2 g extract

↓ taken up in diethyl ether 100 ml, to which was added 400 ml petroleum ether, was adsorbed onto 10 columns (50 ml/column) of powdered sugar (8 × 36 cm) prepared by pressing, developed with petroleum ether plus 0.5% n-propanol, was allowed to run dry.

chlorophyll a and b zones were packed into new tubes, was eluted with ethanol plus petroleum ether. The petroleum ether layers were washed with distilled water, evaporated.

dried chlorophyll a fraction

↓ was dissolved in diethyl ether 50 ml, which was diluted with petroleum ether 200 ml.

5 columns (8 × 36 cm) of powdered sugar (Readsorption)

↓ washed with petroleum ether, developed with petroleum ether plus 0.25% n-propanol, was sacked dry, removed, packed in a new tube, eluted with ethanol plus petroleum ether.

elutriate

↓ was washed with distilled water 50 ml once, extracted with 200 ml portions of 50, 60 70, 80, and 85% methanol to remove colorless, hydrogen-containing substance eluted from the sugar.
The petroleum ether layer was washed three times with distilled water (500 ml), poured in a flask, cooled over dry ice for 24 hrs. The precipated chlorophylls were collected by centrifugation, and dried in high vacuum.

ca. 250 mg

Bacteriochloroplyll

Germine Cohen-Bagire, W. R. Sistrom and R. Y. Stainer Non-sulfer purple bacteria (family Athiorhodceae)
Rhodospirillum rubrum

Culture media (Hutner)

1. Potassium phosphate, pH 6.8 — 1.0 M
2. Ammonium DL-malate, pH 6.8 — 1.0 M
3. Concentrated base:
   - Nitrilotriacetic acid — 10.0 g
   - $MgSO_4$ — 14.45 g
   - $CaCl_2 \cdot 2H_2O$ — 3.335 g
   - $(NH_4)_6Mo_7O_{34} \cdot 4H_2O$ — 9.25 mg
   - $FeSO_4 \cdot 7H_2O$ — 99.0 mg
   - Nicotinic acid — 50.0 mg
   - Thiamin HCl — 25.0 mg
   - Biotin — 0.5 mg
   - Metals "1" — 50.0 ml
   - Distilled water to 1000 ml
4. Metals "1" contains per 100 ml:
   - Ethylenediaminetetraacetic acid — 250.0 mg
   - $ZnSO_4 \cdot 7H_2O$ — 1095.0 mg (250 mg Zn)
   - $FeSO_4 \cdot 7H_2O$ — 500.0 mg (100 mg Fe)
   - $MnSO_4 \cdot H_2O$ — 154.0 mg (50 mg Mn)
   - $CuSO_4 \cdot 5H_2O$ — 39.2 mg (10 mg Cu)
   - $Co(NO_3)_2 \cdot 6H_2O$ — 24.8 mg (5 mg Co)
   - $Na_2B_4O_7 \cdot 10H_2O$ — 17.7 mg (2 mg B)

   A few drops of sulfuric acid are added to retard precipitation.

Stock cultures were maintained as stabs incubated in the light on a solid medium of the following composition: yeast extract, 0.3 g; casamino acids, 0.2 g; agar, 1.5 g; and water 100 ml.

In preparing solution 3, the nitrilotriactic acid was dissolved and neutralized with KOH, after which the rest of the ingredients were added and the pH ws adjusted to 6.7 before making to volume.

In order to prepare 1 liter of the complete medium, 20 ml each of solutions 1, 2 and 3 were mixed and diluted with distilled water, after which 1.0 g of vitamin-free casamino acids was added. A copious precipitate formed when the medium was autoclaved but redissolved on cooling. Casamino acids was replaced by a mixture of L-glutamic acid (0.1% final concentration) and sodium acetate·$3H_2O$ (0.1% final concentration). The medium was perfectly clear and colorless.

Growth condition

Aerobically in the dark: 250 ml Elemeyer flask containing 30 ml of medium, at 30°C, 72 hrs, shaking.

Bacteriochlorophyll

Procedure

12 liters of *Rhospirillum rubrum* cultures

↓ centrifugation

cells

↓ extracted with methanol 500 ml plus diethyl ether 200 ml plus petroleum ether 100 ml
reextracted with methanol 200 ml plus diethyl ether 100 ml.

combined extracts

↓ diluted with water.

layer

↓ washed with water, evaporated.

*purplish gray residue

↓ dissolved in diethyl ether 60 ml, diluted with petroleum ether 300 ml.

solution

↓ adsorbed in 3 columns of powdered sugar (8 × 35 cm).

columns

↓ washed with petroleum ether plus 0.5% n-propanol until the blue-gray zone was carried to the bottom, the zones were repacked into a new tube, eluted with petroleum ether plus ethanol.

elutriate

↓ diluted with diethyl ether, washed twice with distilled water.

3 columns of powdered sugar, with petroleum ether plus 0.75% n-propanol as wash liquid

↓ eluted with petroleum ether plus ethanol.

elutriates

↓ washed with distilled water.

crystals

↓ the suspension was cooled with carbon dioxide, crystals were collected by centrifugation, recrystallized from chloroform by addition of petroleum ether.

ca. 90 mg

Bacteriochlorophyll

Procedure

cells

↓ centrifugation
washed and suspended to about 30% (V/V) in 10 mM HEPES buffer, pH 7.5.
Disrupted the cells by passing the suspension twice through a French pressure cell at about 20000 psi.
Centrifuged the extract at 12000 ~ 15000 G for 30 mins to remove debris.

supernatant (clean blue extract)

↓ Centrifuged at about 20000 G for 60 mins.

jellylike pellet (clean deep blue)

Discussion

The some general problems are taken up at first. Especially the purity, effectiveness, and influence of substances or compounds used for the purification procedure were reported.

Recently, the preparation (purification) methods are improving on the bases of the strict concept. For example, it is considered that the buffer action is most important factor in buffer solution. And the stronger of the buffer action, the better of the buffer factor. However, recently, the ionic strength was considered in the stead of pH. So, it is considered that the selection of the buffer solution was tended to depend on the neighbor less pKa. Moreover, it was emphasized that the very small amount of chemical compound of buffer action was absorbed on the chromatographic column. Then the characteristic of the column was changed with this unexpective adsorption. Moreover, the above-described concept is applied to cell membrane. The following questions are rised up here. The used buffer chemical compound changes the original characteristics of membrane. Then, the characteristic of apoprotein has to be changed.

On the other hand, the very small amount of buffer chemical compounds absorbs the ultraviolet. Fundamentally, the buffer chemical compounds are eluated with the buffer solutions, the chemical solutions or solvents completely. In the fact, the experimental results were discussed. So, the performance of this experiment was carried out on this viewpoint. (1) the pigment for the column is exposed less to air, oxygen, than thin layer or paper chromatography procedure. (2) the suitable adsorbent does not alter the characteristic of the pigment. (3) the dry pressing method is better than slurry method, because the column does not shrink or channel as is sucked dry. Moreover, the usual buffer solution, phosphate buffer, was not used. So, Mops-KOH buffer solution was used in the stead of phosphate buffer solution. The Mops-KOH buffer solution did not show the absorption in ultraviolet. This point is important considerable point. Such consideration induces this improved experimental method. The experiment of Mops-KOH buffer solution might be published on other chance since the experiment of HEPES buffer solution was published on this paper.

Chlorophyll a:

In this case, the used adsorbent had to be purified before this chromatography isolated the pure objective compound. Because, the desorbed substance was necessary. Then the adsorbent, sugar, was purified at first step like the above description. In this isolation procedure, the contamination or entrance of impure unobjective material should be avoided near absolutely even a very small amount. Next, in the case of extraction, when the petroleum ether layer was evaporated, this evaporation was carried out at a comparative low temperature. If it is done at comparative high temperature, the evaportion rate increases, but the material changes to high viscous state and to unnatural state. Then it is not so easy to dissolve or becomes denatured state partially. On the other hand, it was done at comparative low temperature, the material

changed to film state. It was not so difficult to dissolve homogenously, of course, the evaporation rate decreased. Also, this experimental object is the analysis of molecular structure, so the highest pure and natural state should be obtained. The molecular structural change should be avoided completely.

Next, the adsoprtion column chromatography is suitable to isolate a relative large amount of chlorophylls. And so-called pigments are able to expose to air or oxygen using column as less as possible. The separation which the adsorbent is used in column is more faster than the separation which the adsorbent is used in other form, thin layer, in much amount of sample case. But the column is limited to the use of one-way flow of the developing liquid. And some different adsorptive substances were employed for the separations of chlorophylls in columns. Some adsorbents, sucrose, starch, cellulose, celite, silica gel, Sephadex LH-20, polyethylene, polypropylene, Kel-F300, polyamide, or cellulose + dimethylformamide, celite + 7% Wesswon oil, which are reported employ many different developing liquids. Sucrose, starch, and cellulose were tried to estimate these effect. The results except sucrose may be reported at next time. This column was simple adsorbent and not so complex developing liquid for obtain high pure substance. And it was nonreactive to alter the chlorophylls. Most of adsorbents are effective by adsorption except for polyethylene, polypropylene, polychlorofluorocarbon (Kel-F300). Sucrose is dependable adsorbent, is tested for separation of chlorophylls. The column was about 0.5 cm in diameter for the analytical studies, and was 8 ~ 10 cm in diameter and 36 ~ 38 cm long for the preparation work. And its column was not complete to separate all materials. Also, this column did not separate all pigments, notably the chlorophyll c, from each other, non-sorbed carotenes, isomeric xanthophylls, lutein, zeaxanthin were not resolved. Then, as the separation of chlorophyll a was the object, its procedure was useful. Of course, isotopically modified chlorophylls from the ordinary chlorophylls, chlorobium chlorophylls are served with this column in spite of its high resolving power. And columns of adsorbent are formed either by pressing the dry powder into a tube or by pouring a slurry of the adsorbent and a wetting agent into the column. The dry pressing method is superior to the slurry method, because the columns do not shrink or channel when sucked dry.

Buffer action:

In the study of biological reactions; it is important to maintain a constant, but often exceedingly low, concentration of hydrogen ions. For instance, when the pH of reaction medium should be maintained at 8.0 ± 0.1 the permissible range of hydrogen ion concentrations lies between 1.25×10^{-8} and 8.0×10^{-9} M, a range of only 4×10^{-9} M. Yet all too frequently the reaction being studied produces or consumes, many thousands of times this amount of hydrogen ion. Even in systems which in theory neither produce nor consume hydrogen ions, unintentional gain or loss of atmospheric CO_2 or minor side reactions can cause disastrously wide fluctuations in pH. Moreover, biological processes associated with the movement of ions across membranes are discovered constantly. Obviously local pH changes occur whenever the movement of cations across a membrane does not precisely equal the movement of anions.

On the other hand, some buffers do not form complexes with metal ions whereas others, such as phosphate, tend to precipitate polyvalent metals and still other buffers form soluble complexes with metal ions, lowering and stablizing the concentrations of free Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{3+}, and Cu^{2+} etc. These latter hydrogen ion buffers can also be used as metal ion buffers.

Until recently the evaluaton of buffers was difficult because so few were available for comparison, especially range pH 6-9. The great popularity of tris (hydroxymethyl) aminomethane (Tris) was a measure of the shortcomings of alternatives since Tris itself is far from idea in most situations. Accordingly, Good et al.[3] undertook to design and prepare a number of new buffers most of which are now commercially available. HEPES which is in many ways an ideal buffer, interfers with the Folin protein assay. Thus it is obvious that buffers must be tailored to the needs of the system to be studied. Tris buffer displaces the pH curve of electron transport and phosphorylation in chloroplasts by almost a pH unit when compared with other buffers (which all give the same pH optimum) and in electron transport of chloroplasts uncoupled by the removal of coupling factor.

HEPES: N-2-hydroxyethyl piperazine-N^1-ethanesulfonic acid, pK_a 7.55. It does not bind Mg^{2+}, Ca^{2+}, Mn^{2+}, or Cu^{2+}. And one of the best general purpose

buffers available for biological research. It has been used to advantage in tissue culture, in oxidative phosphorylation studies with plant mitcondria, in protein synthesis studies with cell-free bacterial systems, in photophosphorylation studies, and in studies of CO_2 fixation by isolated chloroplasts.[4] Buffer are typically used in massive amounts, the importance of purity cannot be overemphasized.

Tris·HCl buffer was made from commercial guaranteed pure grade reagents. But the purifiation procedure was abbreviated. Because this reagent has been used in other many experimental cases.

Bacteriochlorophyll:

Good et al.[3] and others[5~11] reported on the various viewpoints of characteristics of buffers. (1) these buffers should have maximum solubility in water and minimum solubility in other solvents. To keep the concentration of potentially harmful buffer as low as possible in cellular organella, buffer should pass through membranes with difficulty, if at all. (2) ions should be as few as possible. If specific ions or ionic strengths are required, appropriate salts can be added. (3) the pKas of the buffers should be influenced as little as possible by buffer concentration, temperature, and the ionic constitution of the medium. (4) if the buffer form complexes with metals, these complexes should be soluble and the binding constants should be known. (5) the buffers should resist enzymatic and nonenzymatic alternations, and they should not resemble enzyme substnaces enough to act as analog inhibitors. Neither should they react with any metabolite or with any component of the medium. (6) they should not absorb light at wavelengths longer than about 230 nm less their presence interefere with spectrophotometric determinations. (7) they should be easy to prepare and to purify. Ease of purification is very important, since biological system can be extremely sensitive to minute traces of some kinds of impurities.

The surface active reagent which was used generally, Triton X-100, shows absorption maximum at 275 nm. The absorbance near 280 nm is an indicator of protein content, it can also reflect other substances, notably bacteriochlorophyll and Triton X-100. The bacteriochlorophyll in ether shows a peak[12] at about 260 nm. One of the useful traits of dimethyl laurylamine oxide is that it does not show an absorption band near 280 nm, so it does not interfere with protein estimation. A clear suspension is regained when detergent is added back. Limiting detergent concentrations for this effect depend strongly on the ionic environment and have not been well characterized.

In the case of photochemical reaction center, the compositions of the reaction center preparations made with Triton-100[2] have been published, with lists of the ratios of certain components (quinones, cytochromes, metals) to P870. But the preparations made with this surface active reagent may contain far less of these components, it is hoped that further analysis may show what meaning should be attached to the idea of photochemical reaction center particle, and what structural relationship exists between the photochemical reaction centers and the associated systems of living cell: light harvesting system; electron transport and phosphorylatings machinery. Indeed, the surface active reagent role influences of properties the cell membrane. And as the results the particle size, molecular weight, or particular structure may be depended upon it. Then, cycle electron transport system may be detected like the above description.

Conclusion

This chlorophyll a, isolated and purified from fresh spinach with usage of purified reagents, solvents and adsorbent that were at the highest grade, was considered as one standard sample from these many experimental results. Recently, as the estimation of purification grade from one physical determination (for example, macro X-ray diffraction analysis) became be not sufficient, the several spectroscopic determinations were required to recognize the purification, molecular structure and properties except micro electromagnetic wave diffraction of super microanalytical grade.

This bacteriochlorophyll isolated and purified from *Rhodospirillum rubrum* culture with adsorbent column and high purified reagents, solvents and adsorbents was determined to analyse some properties and molecular structure by the spectroscopies. And the effect of buffer of HEPES was determined against Tris·HCl buffer.

Especially, these purifications were performed with a sufficient attention by the improved method. Under temperature control system, several physicochemical properties of chlorophyll a were determined with ultraviolet

absorption spectroscopy and resonance Raman spectroscopy by the improved cells.

Summary

First, chlorophyll a was isolated and purified from fresh spinach with purified adsorbent column and purified solvents and reagents by the partial improved method. Several physicochemical properties were detected with ultraviolet, infrared and resonance Raman spectroscopies. Infrared and far-infrared absorption spectroscopies were used in usual method and in the film method (hydrated⇄deuterated) that the author developed for infrared absorption. The improved cells were used for resonance Raman spectroscopy. Many determined absorption bands were tried to assign as possible.

This purified sample is considered as one standard sample from these experimental results and is used to determine other spectrospecificities related to molecular structure.

Second, bacteriochlorophyll was isolated and purified in the culture process of *Rhodospirillum rubrum* with purified reagents and solvents by the partial improved method. In the purification process, HEPES buffer solution was used to test the solution effect in the stead of Tris·HCl buffer solution. The effect was detected. Several physicochemical properties were determined with ultraviolet and infrared absorption spectroscopies under usual condition and resonance Raman sepctroscopy under the limited condition using the improved cells. Many infrared absorption bands were tried to assign as possible. This sample is thought as one noticeable sample and is used for other determinations related to molecular structure from these experimental results.

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Foot note:

As a pagination for one report was limited by budget, sections of summary, introduction, discussion, conclusion, and experimental and results should be shortened in that order. Morever, the rigorous restriction was one person one contribution one year.

ヘムタンパク質およびそのモデル化合物の共鳴ラマン分光
第　1　報
クロロフィルムａおよびバクテリオクロロフィルの精製と一部の性質

堀　津　圭　佑

（平成３年９月26日受理）

第１，クロロフィルａが新鮮なホウレンソウから精製された吸着剤カラムと精製された溶媒と試薬で部分的改良された方法により単離・精製された．いくつかの物理化学的性質が紫外，赤外，遠赤外，共鳴ラマン分光により検出された．赤外吸収分光は通常の方法と著者が赤外吸収に対し開発した薄膜法（H化⇆D化）に用いられた．改良セルは共鳴ラマン分光のために用いられた．多くの測定吸収帯は可能の限り帰属を試みられた．

この精製試料はそれらの実験結果から一標準試料として考えられ，また分子構造関係の他の分光学的特性の測定のために用いられる．

第２，バクテリオクロロフィルは *Rhodospirillum rubrum* の培養過程で精製された試薬と溶媒を用い部分的改良された方法により単離・精製された．この精製過程でHEPES緩衝溶液がTris・HCl緩衝の代りに溶液効果を試験するために用いられた，その効果が検出された．いくつかの物理化学的性質が通常の条件下の紫外と赤外吸収分光および改良セルを用い限定条件下の共鳴ラマン分光により測定された．多くの赤外吸収帯は可能の限り帰属を試みられた．この試料は一重要試料と考えられ，またそれらの実験結果から分子構造関係の他の測定のために用いられる．

教養部　生物学第１研究室