THE ISOLATION OF THE GREEN ROD PIGMENT OF THE FROG, *RANA CATESBEIANA*

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1. Introduction

Green rods account for 8% of the total rod population in a frog retina (*Rana temporaria*) and absorb the blue light [1]. The λ_{max} of green rod pigment was determined to be 430–440 nm by the two steps partial bleaching analysis of the extract from outer segments with digitonin [2–4]. The protein of green rod pigment may be like the cone pigment because green rod pigment regenerates more quickly than red rod pigment, rhodopsin, and is destroyed by hydroxylamine [3–5]. However, the properties of green rod pigment was not studied sufficiently because the extract of outer segments contained green rod pigment below 10%. Here, we report the separation of green rod pigment of bullfrog and some properties of this pigment.

2. Materials and methods

Rod outer segments were isolated from dark-adapted bullfrog (*Rana catesbeiana*) retinas by shaking in 0.32 M sucrose under dim red light. Outer segments were purified by discontinuous sucrose density gradient centrifugation [6]. Visual pigments were extracted from the purified outer segments with 2% digitonin solution (20 mM Tris–Hcl (pH 7.5)) and applied on a Concanavalin A–Sepharose 4B column (diam. 1 cm, length 20 cm, Pharmacia). Visual pigments adsorbed on the column were washed by 0.2% digitonin solution (20 mM Tris–HCl (pH 7.5) then eluted by adding 20 mM α-methyl-D-mannoside to the solution (flow rate = 1 ml/30 min).

Green and red rod pigments were measured by the two steps partial bleaching analysis at 15°C [2]. The ΔA_{500} between before and after the first bleaching ($\lambda > 600$ nm, 2 h) corresponds to the amount of red rod pigment and the amount of green rod pigment was measured by the ΔA_{430} between before and after the second irradiation ($\lambda > 500$ nm, 1 h).

The molar extinction coefficient (ϵ_{max}) was determined by the method in [7] using a value of ϵ_{max} = 51 600 for all-*trans* retinal oxime.

Molecular weight was determined by SDS–polyacrylamide gel electrophoresis [6]. CD spectrum was determined with a JASCO J-20 recording spectropolarimeter below 15°C.

3. Results and discussion

The extract from outer segments with 2% digitonin contained 8.6% green rod pigment of total pigments. As shown in fig.1, green rod pigment was eluted earlier than red rod pigment, rhodopsin. In the earlier 4 fractions, green rod pigment accounted for

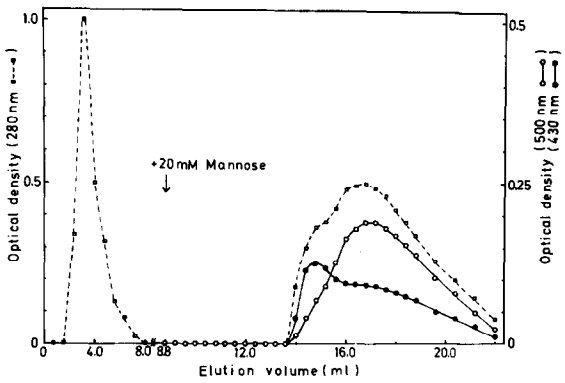

Fig.1. Chromatogram of frog visual pigments in 0.2% digitonin (20 mM Tris–HCl (pH 7.5) on Con A–Sepharose 4B. The A_{280} (□---□) is shown in the scale of the left abscissa. The A_{430} (●——●) and A_{500} (○——○) are shown in the scale of the right abscissa.

56–75% of total pigments and the later fractions were ~100% of rhodopsin. This result indicates that green rod pigment is glycoprotein like rhodopsin and that the structure of carbohydrate chain of green rod pigment is different from that of rhodopsin. Green rod pigment was able to be separated by the chromatography of DEAE-cellulose column, or by salting out of ammonium sulfate, though less efficiently. This suggests that the nature of green rod pigment protein is somewhat different from that of rhodopsin.

Fig.2(a) showed the absorption spectra of the fraction rich in green rod pigment and the fraction of rhodopsin. Fig.2(b) showed the difference spectrum between two curves in fig.2(a) which corresponds to 100% of green rod pigment. The λ_{max} of green rod pigment was 430 nm. Our preparation of rhodopsin had λ_{max} at 502 nm, which indicates that the chromophore of rhodopsin is retinal$_1$ [8]. Therefore, it is reasonable that green rod pigment studied here has retinal$_1$ as its chromophore. The studies on green rod pigment of retinal$_2$ is in progress with the experiment of regeneration from retinal$_2$. The ϵ_{max} values of green rod pigment and of rhodopsin were 35 000 ± 1000 and 38 000 ± 900, respectively. SDS electrophoresis of 75% green rod pigment fraction showed a single band located at the same position as that of rhodopsin, 37 000. This indicates that molecular weight of green rod pigment is almost the same as that of rhodopsin. The λ_{max} of green rod pigment of various frogs were reported 430–440 nm [2–4,9,10], and the ϵ_{max} of it 32 700–40 000 [3,11]. These differences may be derived not from the species of frog but from the method of determination of them, microspectrophotometry or measurement of the extracted mixture containing only 5–10% of green rod pigment.

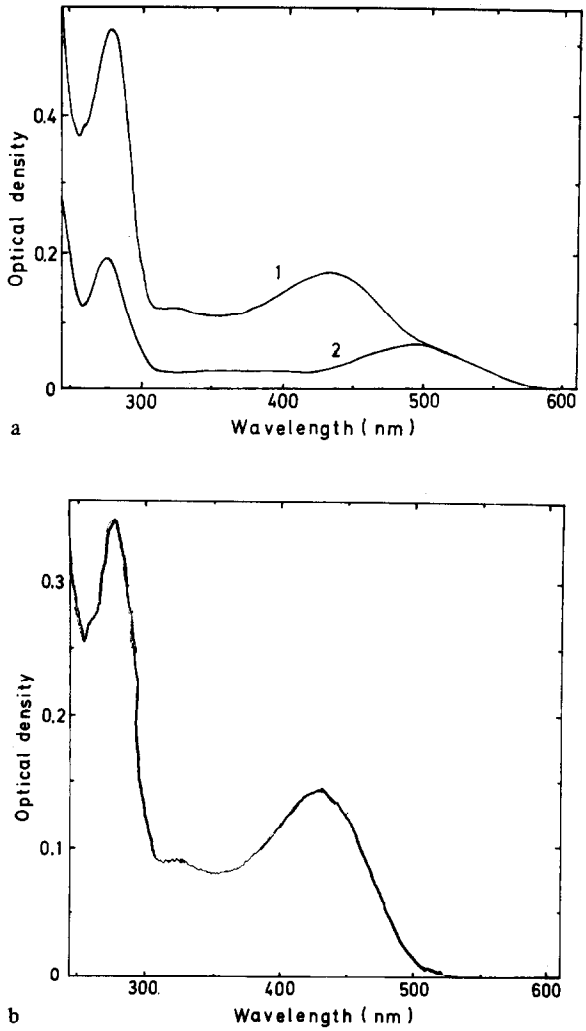

Fig.2(a). Absorption spectra of green rod pigment rich fraction (1) and rhodopsin fraction (2) obtained by the Con A–Sepharose column chromatography. (b) Absorption spectrum of green rod pigment determined from the difference spectrum between (1) and (2) in (a).

Fig.3 showed the CD spectra of green rod pigment and red rod pigment, rhodopsin. Green rod pigment had two positive CD bands (α-band, ~430 nm; β-band, ~310 nm) in the visible region and a negative

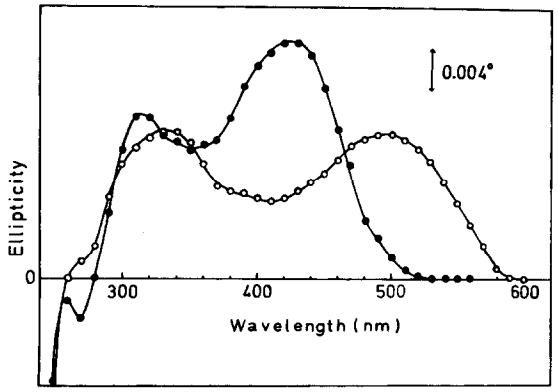

Fig.3. CD spectra of green rod pigment (•——•) and red rod pigment (○——○) in 0.2% digitonin solution (20 mM Tris–HCl (pH 7.5)). The spectra were represented in the scale of ellipticity per maximum absorbance = 1.0 (degree . cm). CD spectrum of green rod pigment was measured after irradiating the 75% green rod pigment fraction with orange light ($\lambda > 600$ nm) for 2 h at 15°C.

γ-band (~270 nm) in the near ultraviolet region. Green rod pigment had a larger α-band CD (molar ellipticity $[\theta]_{430}$ = 81 000) than that of rhodopsin ($[\theta]_{500}$ = 55 000). The CD strength of visual pigment is affected by the detergent concentration of the solution and by the amount of binding lipid with protein [12]. The CD spectra in fig.3 must be close to those of native pigments in membrane because the amount of binding lipid was ~40 mol/mol rhodopsin. The CD band of green rod pigment in the near ultraviolet region was different from that of rhodopsin. The CD band in this region is partly derived from aromatic residues in protein and from S–S linkage. The results of CD measurement suggest the structural difference of protein part of green rod pigment from that of rhodopsin although molecular weights of the 2 pigments were the same.

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