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Hypsochromic Shifts in Retinochrome Absorption Spectra in the Presence of Nitrate

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The absorption wavelength of the protonated retinal Schiff base can be controlled by the surrounding environment. An external anion is related to fine adjustment of the absorption wavelength. The addition of anion to retinochrome solution caused blue shift in spectra. The increase of the shift was dependent on the ion concentration. The large shift value was obtained as 20 nm at the saturated concentration of nitrate. The shift intensity for the nitrate addition exceeded that of chloride. Seemingly, it depends on the ionic strength or lyotropic character of the anion. However, neither of sulphate nor gluconate ion showed remarkable blue shift. These phenomena were accounted for with (1) delocalization of the positive charge in the conjugated polyene system, (2) ionic bonding strength between the counter ion (glutamate) and the proton, and/or (3) interaction of the added anion with the proton on Schiff base.

Key words : retinochrome, hypsochromic shift, Schiff base, lyotropic, nitrate, chloride

INTRODUCTION

Restoration of visual ability is a complementary process of vision. The regeneration of rhodopsin means conversion of all-trans to 11-cis retinals with isomerase. For examples, the two retinal isomerases have been characterized so far, retinochrome and photoisomerase of honeybees [1,2]. In this report, retinochrome was focussed for elucidation of the relationship between the structure and functions. Retinochrome has been found in the photoreceptor cells of cephalopod [1]. The clarified and mechanistic schemes of the regenerating process are described that the chromophore of retinochrome is all-*trans* retinal, which is covalently bound through a protonated Schiff base linkage to a lysine residue [3].

The structure of retinochrome also consists of a

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transmembrane containing seven-spanned helices [4]. However, the remarkable difference for the spectral properties is appeared in the dependency relation to addition of salts [5]. Here, we report the hypsochromic shift in the addition of salt to the squid retinochrome.

MATERIALS AND METHODS

Preparation of Retinochrome. Retinochrome was isolated from retinas of dark-adapted squids (*Todarodes pacificus*) as described in the known method for extracting retinochrome [6].

Sample Preparation for Salts Effect. To analyze the salts effect for the spectrum of retinochrome, 67 mM phosphate buffer (pH 6.5) 400 μl containing each salts to be tested were directly added to retinochrome extract aliquots 100 μl on ice. Then the mixture was incubated for 5 min at 0 °C.

Spectroscopy. All absorption measurements were performed with a Hitachi U-4000 spectrophotometer. The sample was placed in a quartz black cell (1-cm path length). The sample chamber was purged with N_2 gas to prevent condensation of water. To maintain the sample temperature at 0 °C, a cell holder was cooled prior to the measurement with a circulator (Tokyo Rikakikai).

RESULTS AND DISCUSSION

The changes of the absorption spectra with nitrate are shown in Figure 1. The profile indicates blue shift with increase of the concentrations of sodium nitrate. Apparently the shift gave a biphasic profile. However, the detailed analysis revealed early and moderate formations of complex. As a while, the difference spectra based on the nitrate-free spectrum showed two regions; minor non-isosbestic and major isosbestic regions. The former was appeared in the initially adding state, whereas the latter was observed in the subsequent addition. This behaviour was also observed in the case of chloride-addition, where equilibria of the associations have been proposed [7].

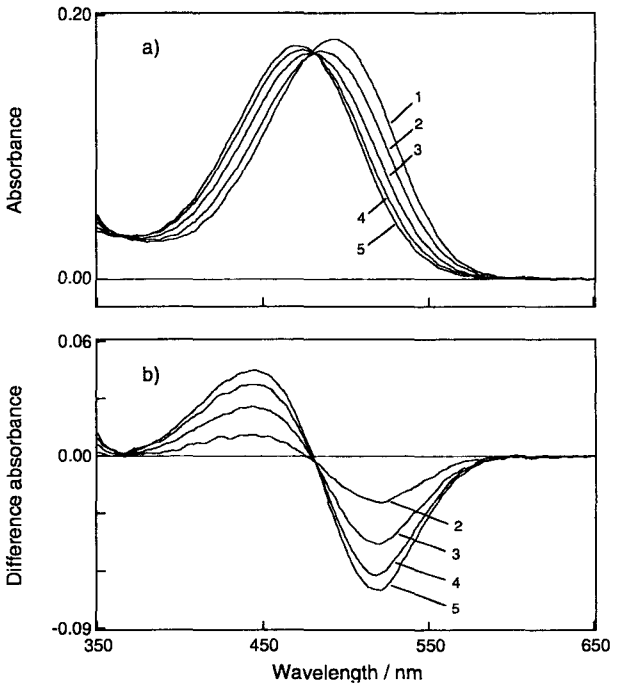

Figure 1. Changes in the retinochrome absorption spectrum upon addition of sodium nitrate at 0°C, pH 6.5. a) Absorption spectral shift of retinochrome with the increase in $NaNO_3$ concentration. The concentrations were adjusted to 0, 0.2, 0.4, 0.6 and 0.8 M for curve 1-5, respectively. b) Difference absorption spectra based on the intensity of curve 1.

To examine the cation contribution to the blue shift in the absorption spectrum of retinochrome, the alkali halides were selected. Each wavelength at the absorption maxima of retinochrome was constant and unchangeable, irrespective of the concentration of the species of alkali nitrate (K^+, Na^+, and Li^+).

For the capability of the shift, the binding of the anion to retinochrome was examined in series of gluconate, nitrate, and halides (Figure 2). The tendency to the capability was ordered as gluconate < Cl^- < NO_3^-.

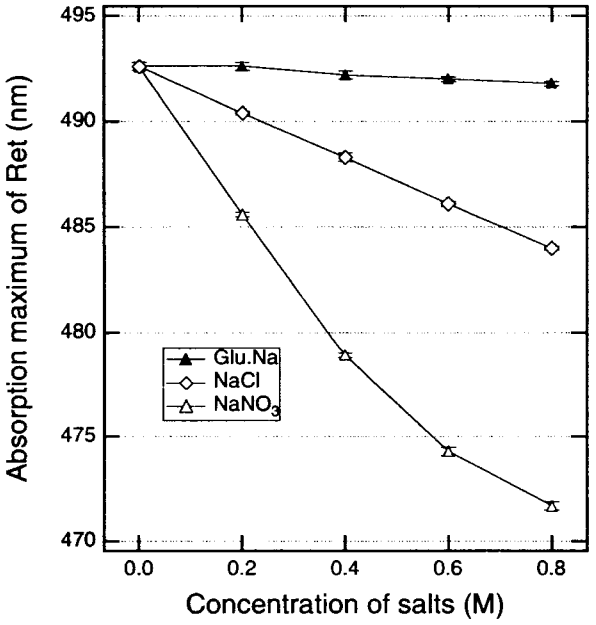

Figure 2. Dependence of λmax of retinochrome on the concentration of various anions at 0°C, pH 6.5. Data points show mean SD of three measurements for each condition.

In conclusion, the absorption spectra of retinochrome were hypsochromically shifted by the addition of anion. The blue shift (20 nm) of the absorption maximum with the salts depends on the inverse relation to the Hofmeister series. The intense shifted value was obtained for nitrate ion at the concentration of 0.2 M.

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