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Stimulatory Effect of Cyanidin 3-Glycosides on the Regeneration of Rhodopsin

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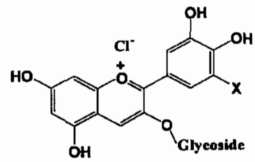
Anthocyanins have been suggested to improve visual functions. This study examined the effect of four anthocyanins in black currant fruits on the regeneration of rhodopsin using frog rod outer segment (ROS) membranes. Cyanidin 3-glycosides, glucoside and rutinoside, stimulated the regeneration, but the corresponding delphinidins showed no significant effect. The formation of a regeneration intermediate was suggested to be accelerated by cyanidin 3-rutinoside. Their effects on the cGMP-phosphodiesterase activity in the ROS membranes were also investigated but found to be negligible. It was concluded that the major effect of anthocyanins in rod photoreceptors is on the regeneration of rhodopsin.

KEYWORDS: **Rhodopsin; regeneration; 11-*cis*-retinal; anthocyanin; black currant**

INTRODUCTION

Rhodopsin, which is a member of the G-protein-coupled receptor family, is localized on the lipid bilayers of disks laminated in the retinal rod outer segment (ROS). On light absorption, 11-*cis*-retinal, the chromophore of rhodopsin, is isomerized to *all-trans*-retinal (*1*). Rhodopsin consequently undergoes further conformational changes through a number of intermediates and triggers a series of reactions in the phototransduction cascade (*2*–*4*). One of the intermediates is metarhodopsin II, which activates >500 transducin molecules per second to amplify the light signal (*5*, *6*). Transducin then activates cGMP phosphodiesterase (PDE) to hydrolyze cGMP. Because cGMP opens a cGMP-gated cation channel, hydrolysis of cGMP by PDE induces the closure of the channel to hyperpolarize the cell. All of the phototransduction components recover to the original inactive state after light. Light-activated rhodopsin is inactivated by phosphorylation, subsequently dephosphorylated, and finally decomposed to *all-trans*-retinal plus opsin, the protein moiety of rhodopsin. Rhodopsin is then regenerated by binding of 11-*cis*-retinal to opsin.

Anthocyanins have been implicated to improve visual functions. Cyanidin, an aglycon of anthocyanin, has been suggested to have effects on the phototransduction cascade by activating (*7*) or inhibiting (*8*) PDE. Anthocyanins are also suggested to have effects on the regeneration of rhodopsin. A mixture of anthocyanins extracted from bilberry is known to improve night vision (*9*). The regeneration of rhodopsin has also been shown to be stimulated by bilberry extract (*10*). Although these studies suggest the effect of anthocyanins on the visual system, the detailed site of the action is not known. In addition, the molecular species responsible for the effects are not known. Because the crude extract contains chemicals other than anthocyanins and there are 15 species of anthocyanins, it has been difficult to identify the active species.



Delphinidin-3-glucoside (D3G), X=OH
Delphinidin-3-rutinoside (D3R), X=OH
Cyanidin-3-glucoside (C3G), X=H
Cyanidin-3-rutinoside (C3R), X=H

Figure 1. Structure of the four anthocyanins in black currant.

Black currant contains four anthocyanins (**Figure 1**): delphinidin 3-rutinoside (D3R), delphinidin 3-glucoside (D3G), cyanidin 3-rutinoside (C3R), and cyanidin 3-glucoside (C3G). In our previous studies, we successfully isolated and purified the four anthocyanins from black currant as crystals (*11*) and showed that they, as well as those from bilberry, have an improving effect on dark adaptation in humans (*12*).

In the present study, using the four forms of the black currant anthocyanins, first we examined the effect of anthocyanins on the PDE activation. Second, we examined their effects on the regeneration of rhodopsin.

MATERIALS AND METHODS

Reagents. Four crystalline components of black currant, D3R, D3G, C3R, and C3G, were purified according to the methods described

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