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

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Anticyanins have been suggested to improve visual functions. This study examined the effect of four anticyanins in black current 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 opsin phosphodiesterase activity in the ROS membranes were also investigated but found to be negligible. It was concluded that the major effect of anticyanins in red phloem sap is on the regeneration of rhodopsin.

KEYWORDS: Rhodopsin; regeneration; 11-cis-retinal; anticyanin; black current

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 GTPase molecules per second to amplify the light signal (5, 6). Transducin then activates cGMP phosphodiesterase (PDE) to hydrolyze cGMP. Because only PDE and the G-protein-coupled receptor, phototransduction cascade, 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 open, the protein moiety of rhodopsin. Rhodopsin is then regenerated by bonding of 11-cis-retinal to regenerate.

Anticyanins have been implicated to improve visual functions. Cyanidin, an aglycone of anticyanin, has been suggested to have effects on the phototransduction cascade by activating (7) or inhibiting (8) PDE. Anticyanins are also suggested to have effects on the regeneration of rhodopsin. A mixture of anticyanins 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 anticyanins 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 anthocyanin and there are 15 species of anticyanins, it has been difficult to identify the active species.

Black current contains four anticyanins (Figure 1): delphinidin 3-glucoside (DRG), delphinidin 3-rutinoside (DRR), cyanidin 3-glucoside (CGH), and cyanidin 3-rutinoside (CRR). In our previous studies, we successfully isolated and purified the four anticyanins from black current in crystals (11) and shown that they, as well as these two bilberry, have an improving effect on daily adaptation in humans (12). In the present study, using the four forms of the black current anticyanins, we first examined the effect of anticyanins on the PDE activation. Second, we examined their effects on the regeneration of rhodopsin.

MATERIALS AND METHODS
Reagents. Four crystalline components of black current, DRR, DRG, CRR, and CGH, were purified according to the method described

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