USING UNO IN THIS PURIFICATION SCHEME RESULTED IN:

- **A REDUCTION IN THE NUMBER OF PURIFICATION STEPS**
- **A LESS COMPLEX PROCEDURE THAN THAT DESCRIBED FOR AN ANALOGOUS PROTEIN**

Purification of the Components of Human Macular Carotenoid-Binding Complex using UNO™ Q and UNO S Ion Exchange Chromatography and the Rotofor Isoelectric Focusing System

Introduction

The macular region of the human retina is responsible for almost all color vision and most of the high resolution visual acuity. This part of the retina, known as the macula lutea, or 'yellow spot', accumulates approximately 200 ng of two closely-related compounds, lutein and zeaxanthin. These compounds are both dihydroxy-α-carotenes which differ in the position of one double bond in the β-ionone ring. Neither of these compounds accumulates in any other human tissues and the macular is virtually devoid of the other major carotenoid, β-carotene.

Isolation and purification of the proteins involved in the active transport and storage of carotenoids has been difficult. Since only primate eyes have maculae, source tissue was extremely limited in quantity and availability. The primate macula is only 5–6 mm in diameter and only about 0.4 mm thick, containing 2–5 mg of total protein. Protease activity is very high in maculae, owing to the high metabolic rate of this tissue, which hampers isolation of intact protein. The half life of the carotenoid-protein complex is about 12 hours and its intense yellow color allows it to be followed during the course of purification.

Purification

Four to six maculae were lysed by sonication in the cold in the presence of detergents and protease inhibitors, followed by centrifugation at 100,000 x g for 10 minutes. One ml of supernatant was applied to an UNO Q-1 column (Bio-Rad catalog # 720-0001) which was monitored at 280 and 436 nm; the latter wavelength being specific for carotenoid absorption (Figure 1). A yellow peak emerged at about 12% B containing 7–8 species

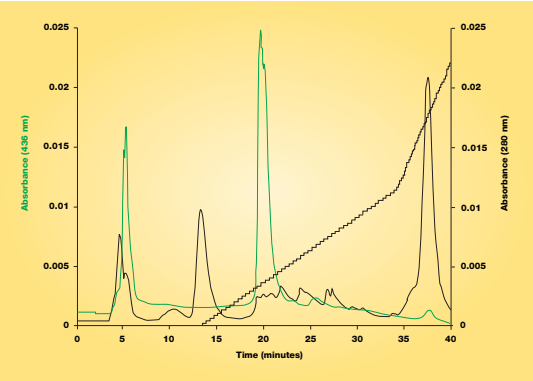

Fig. 1. *UNO Q-1 Chromatography of Maculae Lysate. The column was equilibrated with 20 mM CHAPS in 20 mM Tris-HCl, pH 8.2 (Buffer A) and eluted with a linear gradient of 0–100% Buffer B (Buffer A containing 1 M NaBr). Flow rate: 1 ml/min.*

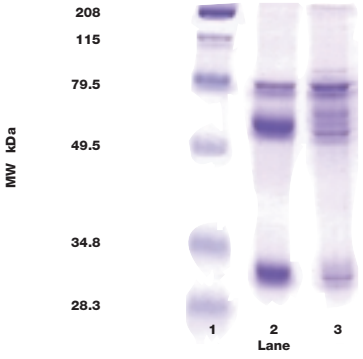

Fig. 2. *SDS-PAGE of UNO-Purified Macular Carotenoid Binding Complex. Lane 1: Bio-Rad Prestained SDS-PAGE Standards, broad range (Bio-Rad catalog # 161-0318); lane 2: fraction eluted at 40% Buffer B from UNO S-1; lane 3: fraction eluted from UNO Q-1 at 12% Buffer B.*

BIO-RAD

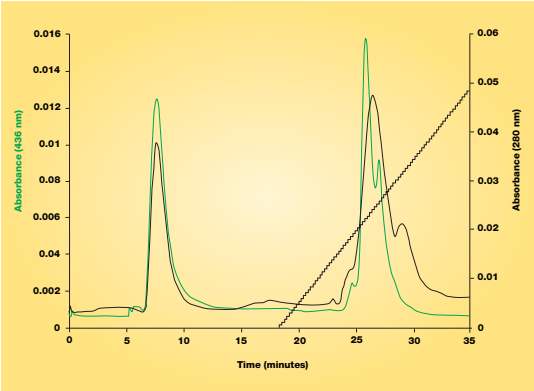

Fig. 3. *UNO S-1 Chromatography of Partially Purified Macular Carotenoid Binding Complex. This column was equilibrated with 20 mM CHAPS in 20 mM ADA, pH 5.8 (Buffer A) and eluted with a 0–100% gradient to 20 mM CHAPS, 1 M NaBr contained in 20 mM Bis-Tris, pH 6.8 (Buffer B). Flow rate: 1ml/min.*

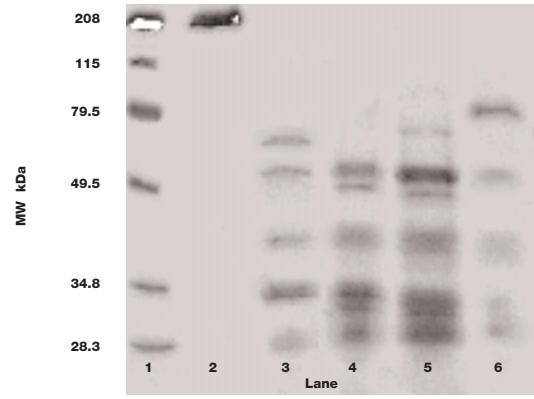

Fig. 4. *SDS-PAGE of Mini Rotofor-Purified Macular Carotenoid Binding Complex. Lane 1: Bio-Rad Standards as in Fig. 2; lane 2: lysis buffer with α_2-macroglobulin; lane 3: pH 5.6 fraction, colorless; lane 4: pH 7.0 fraction, pale yellow; lane 5: pH 7.5 fraction, very yellow; lane 6: pH 8.5 fraction, sharply separated bright yellow.*

by SDS-PAGE (Figure 2, lane 3) with molecular weights ranging from 25–75 kDa. These fractions were pooled, concentrated and applied to an UNO S-1 column (Bio-Rad catalog # 720-0021). The strongly colored peak eluted from this column at 40% B and had about 4 components by SDS-PAGE (Figure 2, lane 2 and Figure 3).

Final purification was performed by isoelectric focusing using the Mini Rotofor Cell (Bio-Rad catalog # 170-2988) and BioLytes covering the pH range of 3–10 (Bio-Rad catalog # 163-1112, 163-1172 and 163-1182). The experiment was scaled up to 28 maculae yielding 2 ml of highly purified, bright yellow carotenoids which were injected into the 18 ml Rotofor core. After focusing for 6 hours at 10 W constant power, 2 colored zones were isolated from fractions with pH values of about 7.0 and 9.0. Fractions were concentrated and analyzed by SDS-PAGE (Figure 4). HPLC analysis indicated that the fraction (Figure 4, lane 6) with a pH of 8.5 contained over 40% of the initial quantity of macular carotenoids and the SDS-PAGE indicated one major protein component.

The 70 kDa protein in this fraction appears analogous to a recently described β-carotene binding protein which required a four step, more complex isolation procedure.[1] The proteins in fraction 6 are currently undergoing sequence studies.

Acknowledgment

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Reference

1 *Rao, M. N., Ghosh, P., and Lakshman, M. R.,* J. Biol. Chem., ***26****, 24455–24460 (1997).*