Isolation and some properties of a carotenoid-protein complex from rat liver

C. Okoh, I. Mychkovsky, and M.R. Lakshman

Lipid Research Laboratory, V.A. Medical Center, Washington, DC USA; and Department of Medicine, The George Washington University, Washington, DC USA

A carotenoid-protein complex has been characterized from aqueous liver extracts of male Wistar rats fed 0.2% (wt/wt) β-carotene, but not from the livers of control rats that did not receive any β-carotene. The carotenoid-protein complex was precipitated from the aqueous 100,000g supernatant fraction between 0 and 50% saturation with ammonium sulphate (AS). Ion exchange chromatography of the AS fraction on a DEAE-sephacel column resulted in the elution of a major yellow fraction that had a characteristic carotenoid absorption spectrum, having a primary peak at 460 nm, with two shoulders at 432 nm and 489 nm. This absorption spectrum was not abolished by treatment with $NaBH_4$, indicating that it is not a flavoprotein. The carotenoid component of the complex was quantitatively extracted with hexane only after denaturing the complex with an equal volume of ethanol or acetone. The organic extract exhibited the characteristic absorption spectrum of β-carotene. The carotenoid-protein complex was successfully reconstituted by incubating the dissociated chromophore with its endogenous apoprotein fraction from rat liver but not with bovine serum albumin. Subcellular distribution of the carotenoid-protein complex showed that 84% of the recovered β-carotene was localized in the mitochondrial and lysosomal fractions, while the nuclear, microsomal, and cytosolic fractions had negligible amounts of β-carotene. These results imply that a significant portion of the carotenoid-protein complex exists in the membrane fraction of the liver cell. Thus, a carotenoid-protein complex may be involved in liver storage and/or transport of β-carotene.
(J. Nutr. Biochem. **4**:569–575, 1993.)

Introduction

B carotene, a photosynthetic pigment, and other carotenoids have recently been reported to exert biological actions in animals distinct from their role as precursors of vitamin A,[1] without the toxicity associated with high vitamin A doses.[2,3] β-carotene is enzymatically converted to vitamin A in the liver and intestines.[4–6] Very little or no intact β-carotene is normally absorbed by rodents, pigs, and chickens[7,8] unless it is fed in substantially large quantities.[9–11] In contrast, humans, ferrets, horses, and certain breeds of cattle are capable of absorbing intact dietary β-carotene and storing quantifiable amounts in the liver and other organs.[7,12,13] In these studies, β-carotene–fed animals had higher concentrations of β-carotene in all the tissues and in serum, relative to controls. The transportation of absorbed β-carotene by low density lipoproteins has been reported.[14,15] However, the mechanism of tissue uptake, storage and transport of intact β-carotene in a mammalian system is not fully understood.

A carotenoid-binding protein has been identified from the cytoplasmic membrane of the heterotrophic cyanobacterium, *Synecocystis sp.*, strain PCC6714.[16,17] Carotenoproteins containing the carotenoids in stoichiometric proportions as prosthetic groups have been isolated from certain crustacea in the form of glycoproteins.[18,19] A carotenoid-protein complex was isolated from the pulp of *Mangifera indica*.[20] So far, no carotenoid-protein complex has been reported for mammals. The inability to characterize such a protein complex from mammalian tissues so far has probably been due to formulating a proper aqueous medium for the extraction of such a carotenoid-protein complex.

In this paper, we present evidence for the partial

This work supported by NCI grant CA39999.
A preliminary report of this work was presented at the FASEB meeting in 1990.
Address reprint requests to Dr. Lakshman at the Lipid Research Laboratory, V.A. Medical Center, Washington, DC 20422 USA.
Received October 8, 1992; accepted January 6, 1993.

characterization of a carotenoid-protein complex from fresh livers of β-carotene–fed male Wistar rats.

Methods and materials

β-carotene feeding

Male Wistar rats (NCI, Bethesda, MD USA) weighing approximately 200 g were used in this study. β-carotene beadlets containing 10% (wt/wt) β-carotene and placebo beadlets containing no β-carotene were gifts from Hoffmann-La Roche and Co., Basel, Switzerland. The experimental diet was made by mixing 20 g of β-carotene beadlets with 980 g of rat chow (Linguard F. Klein, Co., Inc., Baltimore, MD USA) to give a final β-carotene concentration of 0.2% (wt/wt) in the diet. This diet was fed to the experimental animals for 12 weeks, whereas the control rats were fed regular rat chow supplemented with 2% (wt/wt) placebo beadlets. At the end of the feeding period, all rats were killed by aortic exsanguination under pentobarbital anesthesia (50 mg/kg, IP), and the livers were isolated after perfusion with ice-cold 0.85% (wt/vol) saline. Each liver was analyzed for the carotenoid-protein complex and for β-carotene as described below.

Preparation of aqueous extracts

The "homogenization buffer" (pH 6.5) was made up of the following constituents: 50 mM morpholinoethane sulfonic acid (MES), 1 mM EDTA, 20% glycerol, 0.2% n-octyl β-D-glucopyranoside (OβDG), 5 mM 3-[(3- chloramidopropyl) dimethylammonio-1-propane sulfonate (CHAPS), 0.5% Triton X-100, butylated hydroxytoluene (BHT) at a concentration of 50 μg/mL, 2 mM phenylmethylsulfonyl fluoride (PMSF), aprotinin and leupeptin, each at a concentration of 1 μg/mL. All manipulations were carried out under F40 Gold fluorescent light in an ice bath. Each fresh liver from both experimental and control groups was homogenized with a polytron homogenizer (Brinkman Instruments, Westbury, NY USA) in 20 volumes of the homogenization buffer. This homogenate was centrifuged at 100,000*g* for 1 hr, and the supernatant fraction was saved. The pellet was sonicated with another 10 vol of homogenization buffer in a sonicator (Heat Systems-Ultrasonics, Inc., Plainview, NY USA), and the sonicate was again centrifuged at 100,000*g* for 1 hr. This supernatant fraction was combined with the first supernatant fraction and stored at 4° C for further studies.

Isolation of the carotenoid-protein complex

The pooled supernatant fraction from the liver was saturated with 0–50% with ammonium sulfate (AS) and centrifuged at 25,000*g* for 30 min. The yellow pellet fraction was dissolved in 3.5 mL of the homogenization buffer and dialyzed extensively against the "elution buffer," which differed from the homogenization buffer in that only 5% glycerol was included and CHAPS and OβDG were omitted. The dialysate was stored at 4° C for spectrophotometric, chromatographic, and electrophoretic studies and for quantitative analyses.

The ammonium sulphate fraction was further fractionated on a DEAE-Sephacel column (1.5 cm × 25 cm bed; Sigma Chemical Co., St. Louis, MO USA), equilibrated in the elution buffer (homogenization buffer containing 5% glycerol with CHAPS and OβDG omitted). The column was initially washed with two bed-volumes of the elution buffer containing 10 mM NaCl, and then with two bed-volumes of the same buffer containing 100 mM NaCl. Finally, the elution buffer containing 350 mM NaCl was used at a flow rate of 0.4 mL per minute. The carotenoid-protein complex was eluted from the column as a yellow band. The fractions corresponding to the yellow band were pooled and concentrated by the use of a speed vacuum concentrator (Forma Scientific, Inc., Marrieta, OH USA), and stored at 4° C for further studies.

Subcellular distribution of the carotenoid-protein complex

This was investigated by the fractionation of each liver in detergent-free homogenization buffer, pH 6.5, according to the method described by Mayne and Parker.[11] Care was taken to add the appropriate amounts of the detergents to each subcellular fraction to solubilize the protein complex only after it was isolated and subjected to 0–50% AS fractionation. Similarly, for the isolation of the total extractable complex, a portion of each liver was homogenized with the homogenization buffer and the 100,000*g* supernatant fraction was subjected to 0–50% AS fractionation. Each AS fraction was dissolved in a small volume of homogenization buffer and analyzed for β-carotene absorption spectrum. Furthermore, the lipid extract of an aliquot of each AS fraction was analyzed for β-carotene by high pressure liquid chromatography (HPLC) as described below.

Quantitative analyses

Total proteins in the crude AS extract and the DEAE-Sephacel concentrated fraction were measured by the Pierce-bicinchoninic acid method (Pierce, Rockford, IL USA). Total carotenoid concentration of liver tissue was extracted by homogenizing 1.0 g of liver with 20 volumes of chloroform:methanol (2:1, vol/vol) and filtering under vacuum over anhydrous sodium sulfate on a sintered glass filter. For the crude AS extract and the partially purified protein, β-carotene extraction was achieved by shaking 1 mL aliquot with an equal volume of ethanol or acetone before extracting with 8 mL of hexane. The organic extracts were evaporated under nitrogen and redissolved in 1 mL of hexane. β-carotene concentration was assayed by a reversed phase HPLC method, using Gilson HPLC Systems (Gilson Medical Electronics, Inc., Middleton, WI USA). Separation was achieved on a C-18 column (ODS-5 μ; 0.46 × 10 cm) with a mobile phase of methanol-water 97:3 (vol/vol) containing 0.5% ammonium acetate for the first 8 min and 100% methanol thereafter for the elution of β-carotene. Mobile phase was delivered at a flow rate of 1 mL/min and at a pressure of 2000 psi. The detection of β-carotene was monitored with a Kratos Model 783 detector (API Analytical Kratos Div., NJ) at 460 nm. Authentic β-carotene (Hoffmann La-Roche) had a retention time of 11.5 min under these conditions, and, based on the area under the curve for this external standard, the tissue concentration of β-carotene was determined. The recovery of added β-carotene to the liver homogenate was 95 ± 3 (average of five independent analyses). However, no correction has been applied in the reported values. All absorption spectra were monitored in a Shimadzu uv-160 Recording Spectrophotometer (Shimadzu Corp., Kyoto, Japan).

Gel electrophoresis

The yellow fraction that was eluted from the DEAE-Sephacel column was electrophoresed on an 8–12% linear gradient polyacrylamide vertical gel (BioRad Laboratories, CA) in the presence of sodium dodecyl sulfate (SDS-PAGE) essentially as described by Lemmli and Favre,[21] and stained with Coomassie Brilliant Blue.

Release of the apoprotein component of the complex

Four mL of the carotenoid-protein complex was treated with an equal volume of ice-cold absolute ethanol or acetone, shaken gently and left on ice for 30 min, and the mixture was centrifuged at 1300*g* for 10 min. The supernatant (organic) fraction was removed while the pellet (protein) fraction was dissolved in 4 mL homogenization buffer, and the protein was reprecipitated with an equal volume of ice-cold ethanol or acetone as before. This pellet was redissolved in 4 mL homogenization buffer and represented the apoprotein component of the complex.

The chromophore

The organic extracts from the above procedure were evaporated under nitrogen and redissolved in 400 μL acetone. This fraction represented the chromophore of the complex. Both the apoprotein and the chromophore fractions were stored at 4° C.

Reconstitution of the chromophore-protein complex

A 2-mL aliquot of the apoprotein solution (4 mg/mL) in homogenization buffer was mixed with 200 μL of the chromophore solution in acetone and incubated in a 37° C water bath for 1 hr with gentle shaking. As a control, a 2-mL aliquot of bovine serum albumin (BSA) in homogenization buffer (4 mg/mL) was mixed with 200 μL of the chromophore solution in acetone and incubated under identical conditions. The incubation mixtures were dialyzed against elution buffer overnight at 4° C. The dialyzed mixtures were analyzed spectrophotometrically.

Extraction of the chromophore before and after ethanol denaturation

Each of the complexes from the above step was directly extracted once with 6 mL hexane, left on ice for 30 min, and centrifuged at 1300*g*. The hexane phase was saved for analysis while the aqueous solution was mixed with 2 mL ethanol and then extracted once with 6 mL hexane, left on ice, and centrifuged at 1300*g* for 10 min. Ninety percent of β-carotene is extracted from the complex after denaturation with ethanol, and therefore, repeated extraction of the denatured complex with hexane was unnecessary in this experiment. The two hexane phases before and after ethanol denaturation of the complex were analyzed spectrophotometrically.

Results and discussion

As shown in *Figure 1A*, the absorption spectrum characteristic of β-carotene, with peaks at 460 and two shoulders at 432 and 489 nm, was obtained from the 0–50% AS pellet fraction of the 100,000*g* fraction of livers from β-fed rats. Although yellow color was observed in the liver homogenates, supernatant fractions, and the 0–50% AS fraction from control rats, spectrophotometric studies failed to reveal any detectable carotenoid absorption spectrum, even in the AS fraction *(Figure 1B)*. HPLC studies of organic extracts of the experimental livers showed a mean β-carotene concentration of 29.2 nmoles/g liver *(Table 1)*.

Ion exchange chromatography of the AS fraction from the β-carotene–fed rat on a DEAE-Sephacel column yielded a partially purified yellow fraction *(Figure 1C)*. This fraction had a characteristic absorption spectrum of a carotenoid having a primary peak at 460 nm, with two shoulders at 432 nm and 489 nm *(Figure 2A)*. These characteristics are similar to those described for the carotenoid-binding protein of *Mangifera indica* by Subbarayan and Cama.[20] The corresponding yellow fraction obtained from the liver of a control rat failed to show the characteristic carotenoid absorption spectrum *(Figure 2B)*.

Table 1 Subcellular distribution of carotenoid-protein complex in livers of β-carotene–fed rats

Liver fraction	nmoles β-carotene/g	% Distribution
Total liver	29.21 ± 0.72	—
Liver (extractable)	10.07 ± 0.68	100
Nuclear fraction	Not Detectable	—
Mitochondria	5.77 ± 0.69	57
Lysosomes	2.74 ± 1.02	27
Microsomes	0.35 ± 0.08	4
Cytosol	0.13 ± 0.01	1

Subcellular fractions were isolated from the fresh livers of four β-carotene–fed rats, and each aqueous extract was dispersed in the homogenizing buffer containing the detergents and the 0–50% AS pellet fraction was subjected to β-carotene extraction and analysis by HPLC as described in the Methods section. Each value is the mean ± SE.

No carotenoid could be extracted from an aliquot of the carotenoid-protein complex fraction from the DEAE-Sephacel column when shaken with an equal volume of hexane at room temperature in a separating funnel, as evidenced by the absence of a characteristic carotenoid spectrum in the organic extract. This showed that the carotenoids were not present as free pigments and therefore could not be extracted with organic solvents. However, treatment of an aliquot of the same fraction with an equal volume of ethanol or acetone and subsequent extraction with hexane yielded an extract with a characteristic β-carotene absorption spectrum *(Figure 3)*, indicating that the carotenoid in the aqueous extract is most likely the intact β-carotene stored as a protein complex in the experimental livers. These findings support the data from previous investigations, which reported the storage of quantifiable amounts of intact β-carotene in the liver and other organs of rats, chicks, ferrets, mice, and guinea pigs.[7–13]

Significantly, the incubation of chromophore with the intracellular liver apoprotein, as well as with BSA, yielded the characteristic carotenoid spectra as in *Figure 4* (data not shown). However, the chromophore was extracted from the apoprotein-chromophore complex with hexane only after denaturation with ethanol (presence of carotenoid spectrum in the hexane phase only after ethanol treatment). In contrast, the chromophore was easily extracted from the BSA-chromophore loose complex directly with hexane without prior treatment with ethanol. These results strongly support the high

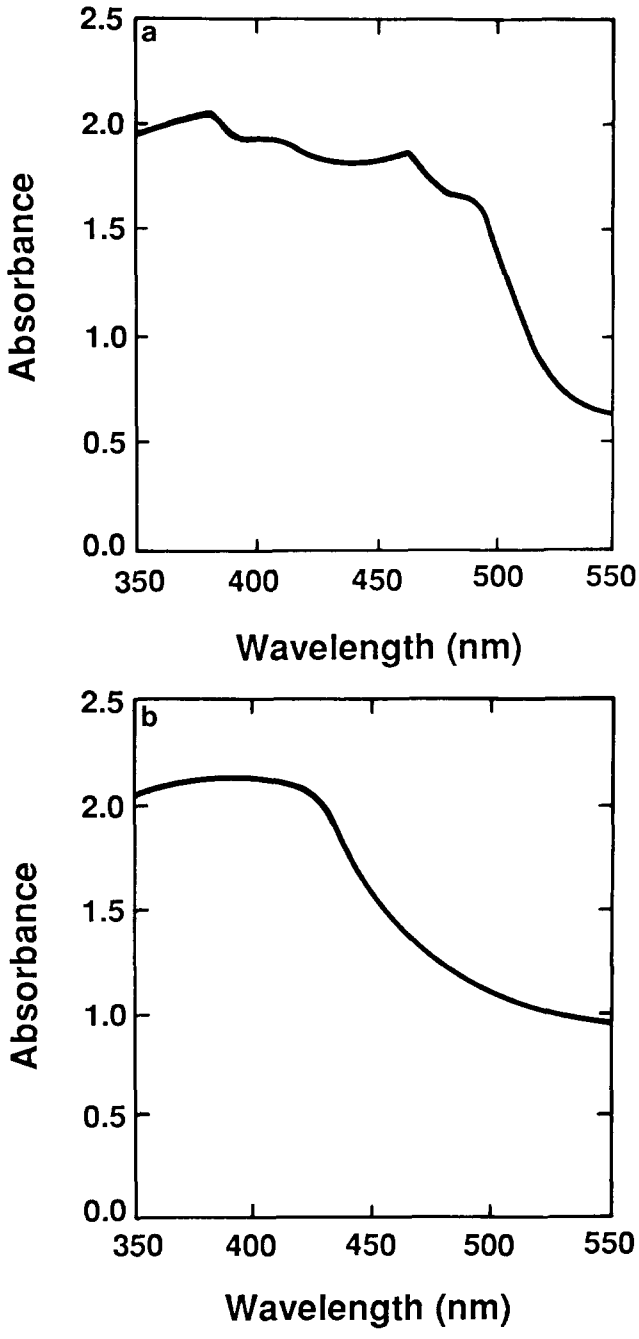

Figure 1 The absorption spectrum in the homogenization buffer of the aqueous extract from the AS pellet fraction of the liver 100,000*g* supernatant fraction from the (a) β-carotene–fed rats and (b) control rats.

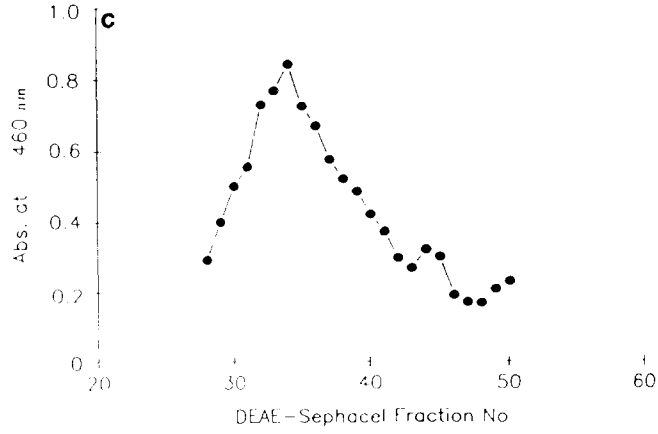

Figure 1c Ion-exchange chromatography of the AS pellet fraction on DEAE-Sephacel column.

specificity of an intracellular liver apoprotein for the formation of a β-carotene–protein complex, whereas the chromophore seems to have low affinity for BSA to form a complex.

The partially purified carotenoid-protein complex was sensitive to temperatures higher than 4° C, prolonged liver storage at temperatures between −20 and −4° C for periods longer than 1 month, presence of proteases, freezing and thawing, and bright light. Each of these conditions caused an initial disappearance of the 432 nm shoulder peak and a gradual hypsochromic shift of the 460 nm peak. The carotenoid-protein complex is fairly stable in the presence of protease inhibitors and high salt concentrations. The inability of sodium borohydride to abolish the carotenoid absorption spectrum of the carotenoid-protein complex *(Figure 4A and 4B)* supports the conclusion that the observed spectrum was due to the carotenoid-protein complex and not to flavoproteins. Flavoproteins are known to lose their absorption spectra on reduction with sodium borohydride.

Subcellular distribution of the complex in the liver of β-carotene–fed rats is presented in *Table 1.* Surprisingly, 65% of the liver β-carotene was still associated with the cell debris that essentially contained unbroken liver cells (light microscopy). Of the remaining β-carotene, 57% and 27% were recovered in the mitochondrial and lysosomal fractions, respectively. The microsomal and cytosolic fractions accounted for only 4% and 1%, respectively, of the recovered β-carotene. Significantly, based on the absorbance of the aqueous extracts of each subcellular fraction at 460 nm, the subcellular distribution pattern of the complex agreed closely with that obtained by HPLC analyses (data not shown). This supports the possibility that β-carotene exists as a protein complex in the liver. A similar pattern of subcellular distribution of β-carotene was reported by Mayne and Parker[11] for high β-carotene–fed chick liver, with the highest amount incorporated into chick liver mitochondria. High carotene content of the mitochondrial fraction of bovine lactating tissue was reported by Patton et al.[22] The role of the mitochondria in cellular processing of β-carotene is yet unclear.

The relatively high absorbance value and β-carotene concentration of the lysosomal fraction may be explained by the fact that β-carotene in human blood is transported primarily by low density lipoproteins (LDL),[14,23,24] which are taken up by the liver and transported to lysosomes. Apolipoprotein B-100 (apoB) is

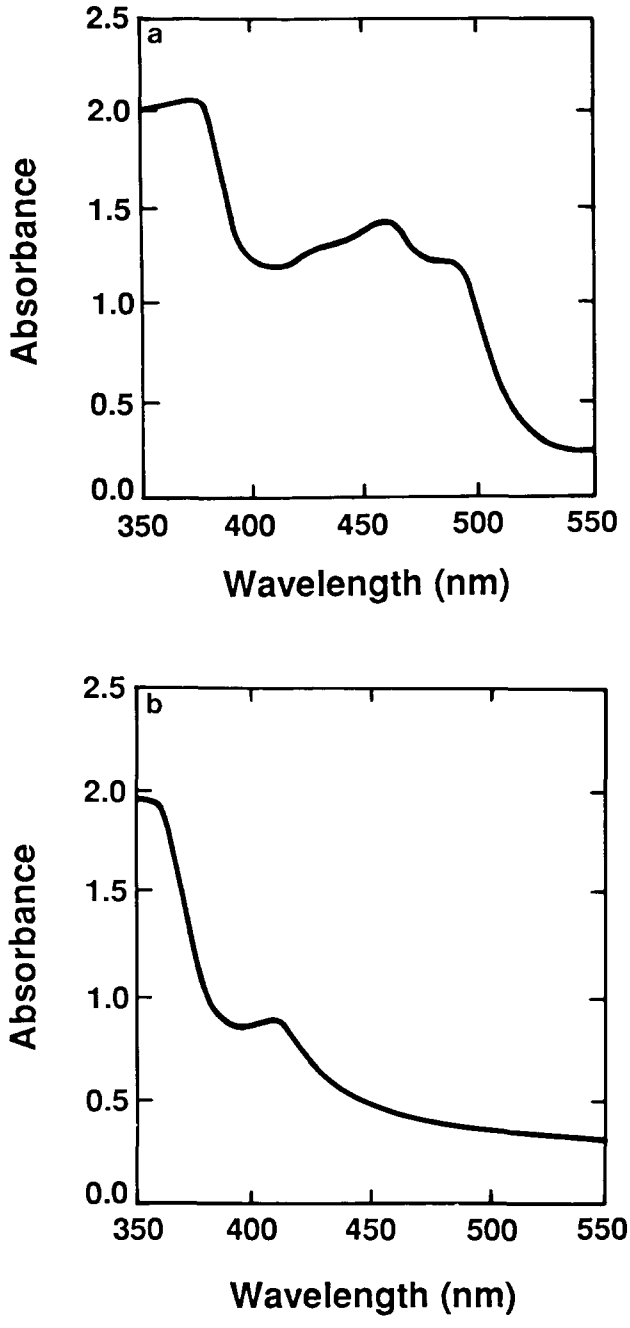

Figure 2 The absorption spectrum in the elution buffer of the aqueous extract of the yellow fraction from the DEAE-Sephacel column of the (a) β-carotene–fed rats and (b) control rats.

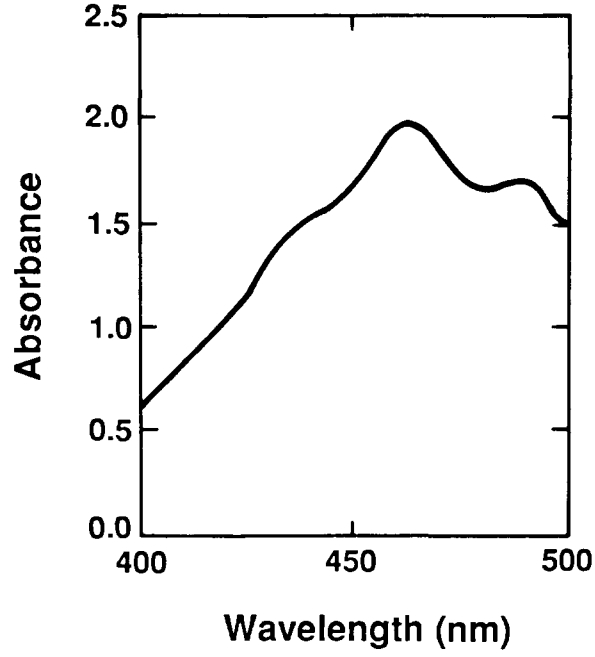

Figure 3 The absorption spectrum in hexane of the lipid extract of the yellow fraction from the DEAE-Sephacel column of the β-carotene–fed rats.

the primary apolipoprotein of LDL and is synthetized by the liver.[25–27] Some investigators have used sedimentation equilibrium or gel permeation chromatography and estimated the molecular weight to be 250,000–275,000.[28,29] Other species of apoB usually observed on 4% SDS-PAGE are apoB-74 and apoB-26, with molecular weights of approximately 300,000 and 100,000, respectively.[30] Amino acid composition data have suggested that these two species are fragments of apoB-100 and can be produced from LDL by the proteolytic action of the enzyme kallikrein.[31] A carotenoid-carrying lipoprotein (CCL) was isolated from the high density lipoprotein (HDL) fraction of chum salmon serum by a sequential ultracentrifugation technique, DEAE-cellulose, and gel filtration column chromatography.[15] The CCL gave rise to two apolipoproteins having molecular weights of 24,000 (apo-I) and 12,000 (apo-II).

The partially purified carotenoid-protein complex isolated from β-carotene–fed rat liver in the present study, when electrophoresed on polyacrylamide gel in the presence of sodium dodecyl sulfate, gave four major bands with molecular weights ranging between 40,000 and 60,000. Whether this carotenoid-protein complex is related to the reported HDL-derived CCL, any of the known fragments of apoB, or to other retinoid binding proteins[32] remains to be seen. The carotenoid-binding protein identified from the heterotrophic cyanobacterium, *Synechocystis sp.*, strain PCC6714 is a membrane protein,[16] with a molecular weight of 35,000 when solubilized at 0° C, and with a molecular weight of 45,000 when solubilized at 70° C. Whether the carotenoid-protein complex isolated in the present study is similar to the bacterial carotenoid-protein complex remains to be verified. Thus, the findings of this study demonstrate the existence of a carotenoid-protein complex in the fresh livers of β-carotene–fed rats, which may play an important role in liver storage and/or transport of intact β-carotene. Further work should clarify the nature of this protein, its binding affinity for β-carotene and other carotenoids, and its role in cellular uptake and transport.

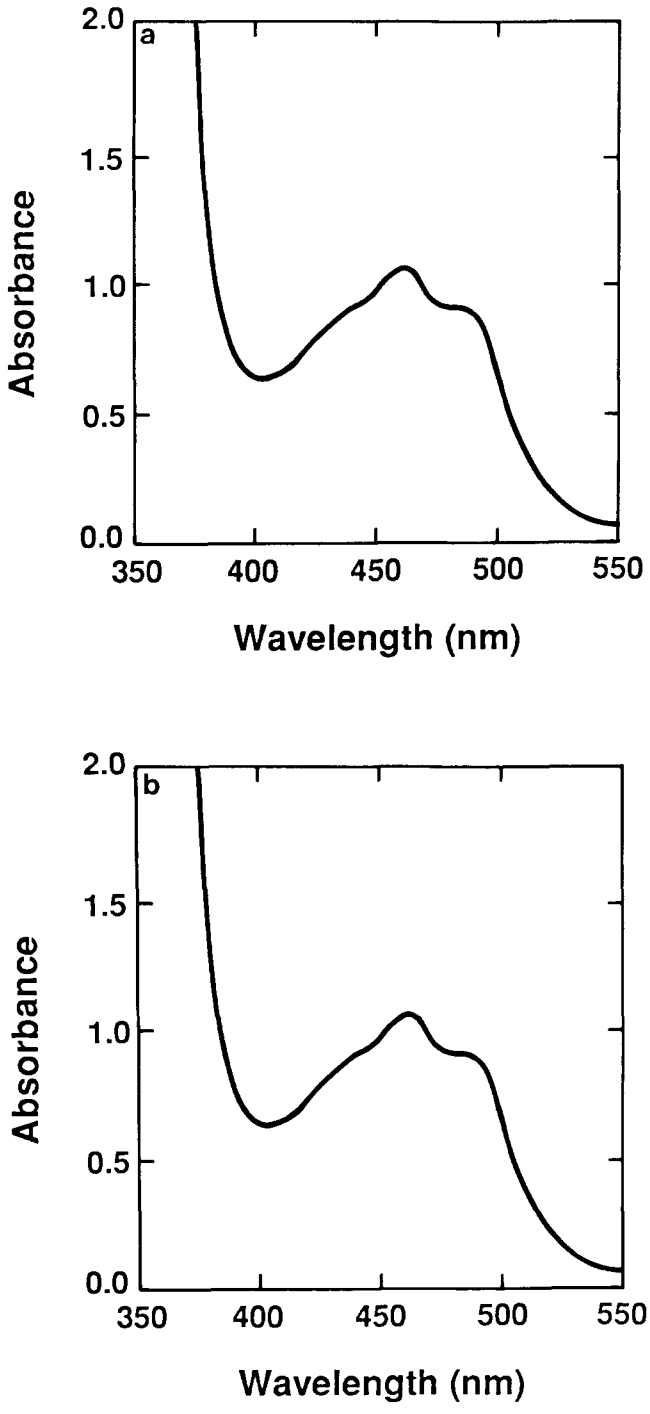

Figure 4 The absorption spectrum of the carotenoid-protein complex (a) before and (b) after treatment with sodium borohydride.

References

1 Benedich, A. and Olson, J.A. (1989). Biological actions of carotenoids in mammals. *FASEB J.* **3,** 1927–1932

2 Burton, G.W. (1989). Antioxidant actions of carotenoids. *J. Nutr.* **119,** 109–111

3 Burton, G.W. and Ingold, K.U. (1984). β-carotene: an unusual type of lipid anti-oxidant. *Science* **224,** 569–573

4 Goodman, D.S. and Huang, H.S. (1965). Biosynthesis of vitamin A with rat intestinal enzymes. *Science* **149,** 879–880

5 Olson, J.A. and Hayaishi, O. (1965). The enzymatic cleavage of β-carotene into vitamin A by soluble enzymes from rat liver and intestine. *Proc. Nat. Acad. Sci. USA* **54,** 1364–1370

6 Lakshman, M.R., Mychkovsky, I., and Attlesey, M. (1989). Enzymatic conversion of *all-trans* β-carotene to retinal by a cytosolic enzyme from rabbit and rat intestinal mucosa. *Proc. Natl. Acad. Sci. USA* **86,** 9124–9128

7 Bondi, A. and Sklan, D. (1984). Vitamin A and carotene in animal nutrition. *Prog. Food Nutr. Sci.* **8,** 165–191

8 Ganguly, J., Mehl, J.W., and Deuel, H.J., Jr. (1953). Studies on carotenoid metabolism XII. The effect of carotenoids on the carotenoid distribution in the tissues of chicken. *J. Nutr.* **50,** 59–71

9 Mathews-Roth, M.M. (1977). The carotenoid content of various organs of animals administered large amounts of beta-carotene. *Nutr. Rep. Int.* **117,** 419–423

10 Shapiro, S.S., Mott, D.J., and Machlin, L.J. (1984). Kinetic characteristics of beta-carotene uptake and depletion in rat tissue. *J. Nutr.* **114,** 1924–1933

11 Mayne, S.T. and Parker, R.S. (1986). Subcellular distribution of dietary beta-carotene in chick liver. *Lipids* **21,** 164–169

12 Blomstrand, R. and Werner, B. (1967). Studies on the intestinal absorption of radioactive β-carotene and vitamin A in man. *J. Lab. Clin. Invest.* **19,** 339–345

13 Ribaya-Mercado, J.D., Holmgren, S.C., Fox, J.G., and Russel, R.M. (1989). Dietary β-carotene absorption and metabolism in ferret and rats. *J. Nutr.* **119,** 665–668

14 Krinsky, N.I., Cornwell, D.G., and Oncley, J.L. (1958). The transport of vitamin A and carotenoids in human plasma. *Arch. Biochem. Biophys.* **73,** 233–246

15 Ando, S. and Hatano, M. (1988). Isolation of apolipoproteins from carotenoid-carrying lipoprotein in the serum of chum salmon. *Oncorhynchus keta. J. Lipid Res.* **29,** 1264–1271

16 Bullerjahn, G.S. and Sherman, L.A. (1986). Identification of a carotenoid-binding protein in the cytoplasmic membrane from the heterotrophic cyanobacterium *Synechocystis* sp. strain PCC6714. *J. Bacteriol.* **167,** 396–399

17 Holt, T.K. and Krogmann D.W. (1981). A carotenoid protein from cyanobacteria. *Biochim. Biophys. Acta* **637,** 408–414

18 Lee, W.L. and Zagalsky, P.F. (1966). The specificity of the carotenoid-protein linkage in crustacyanin. *Biochem. J.* **101,** 9c–11c

19 Cheeseman, D.F., Lee, W.L., and Zagalsky, P.F. (1967). Carotenoproteins in invertebrates. *Biol. Rev.* **42,** 132–160

20 Subarrayan, C. and Cama, H.R. (1966). Isolation and characterization of a carotenoid protein complex from *Mangifera Indica* (Mango). *Indian J. Biochem.* **3,** 225–227

21 Lemmli, U.K., and Favre, M. (1973). Maturation of the head of the bacteriophage T_4. *J. Mol. Biol.* **80,** 575–599

22 Patton, S., Kelly, J.J., and Keenan, T.W. (1976). Carotene in milk fat globules: Observations on origin and high content in mammary mitochondria. *Lipids* **15,** 33–38

23 Bjornson, L.K., Kayden, H.J., Miller, E., and Moshell, A.N. (1976). The transport of α-tocopherol and β-carotene in human blood. *J. Lipid Res.* **17,** 343–352

24 Chen, G.C., and Kane, J.P. (1974). Contribution of carotenoids to the optical activity of human serum low-density lipoprotein. *J. Biochem.* **13,** 3330–3335

25 Kane, J.P. (1983). Apoprotein B: Structural and metabolic heterogeneity. *Annu. Rev. Physiol.* **45,** 637–650

26 Elovson, J., Huang, Y.O., Baker, N., and Kannan, R. (1981). Apolipoprotein B is structurally and metabolically heterogeneous in the rat. *Proc. Nat. Acad. Sci. USA* **78,** 157–161

27 Brown, M.S. and Goldstein, J.L. (1983). Lipoprotein receptors in the liver. *J. Clin. Invest.* **72,** 743–747

28 Steele, J.C.H., Jr. and Reynolds, J.A. (1979). Molecular weight and hydrodynamic properties of apolipoprotein B in guanidine hydrochloride and sodium dodecyl sulfate solutions. *J. Biol. Chem.* **254,** 1639–1643

29 Steele, J.C.H., Jr. and Reynolds, J.A. (1979). Characterization of the apolipoprotein B polypeptide of human plasma low-density lipoprotein in detergent and denaturant solutions. *J. Biol. Chem.* **254,** 1633–1638

30 Kane, J.P., Hardman, D.P., and Paulus, H.E. (1980). Hetero-

geneity of apolipoprotein B: Isolation of new species from human chylomicrons. *Proc. Natl. Acad. Sci. USA* **77,** 2465–2469

31 Cardin, A.D., Witt, K.R., Chao, J., Margolius, H.S., Donaldson, V.H., and Jackson, R.L. (1984). Degradation of apolipoprotein B-100 of human plasma LDL by tissue and plasma Kallikrein. *J. Biol. Chem.* **259,** 8522–8528

32 Chytil, F. and Ong, D.E. (1984). Cellular retinoid binding proteins. In *The Retinoids,* (M.B. Sporn, A.B. Roberts and D.S. Goodman, eds.) p. 90–119, Academic Press, New York, NY USA