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Communication

ChrA Is a Carotenoid-Binding Protein in Chromoplasts of *Capsicum annuum*[1]

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ABSTRACT

Chromoplasts of *Capsicum annuum* var Albino contain a carotenoid-protein complex, which migrates as a brilliant orange band in gels under conditions of nondenaturing electrophoresis. In a second, denaturing separation, the complex resolves into a principal protein (ChrA) of 58 kilodaltons and several minor proteins of 20 to 55 kilodaltons, which may be adventitiously associated. Analysis of Western blots of both one- and two-dimensional gels showed that the principal protein component of the carotenoid complex is ChrA, a protein previously shown to be located specifically in chromoplast membranes. The identification of ChrA as a carotenoid-binding protein appears to be the first instance of a nonthylakoid, carotenoid-binding protein in higher plants.

Chromoplast membranes of red varieties of the sweet pepper, *Capsicum annuum*, contain two abundant proteins, ChrA[5] and ChrB (4). ChrA, which migrates with a molecular mass of about 58 kD, appears only in the final stages of color development of the fruit. ChrB, which is about 35 kD, accumulates early and remains through ripening (10). The proteins are abundant in chromoplasts of red varieties of *C. annuum*, but would not appear to be components of chromoplasts generally, since ChrA was not found in yellow peppers and neither was detected in tomato.

In an effort to identify functions for ChrA and ChrB, we noted that red varieties of *C. annuum* carry the y^+ gene (5) and contain several carotenoids not seen in green or yellow fruits (1, 14). Capsanthin, a ketocarotenoid, is particularly abundant in ripe fruits carrying y^+. We decided to test the possibility that one or both of the chromoplast-specific proteins might play a role in the accumulation of these carotenoids.

MATERIALS AND METHODS

Plant Material

Capsicum annuum L., vars Albino and Emerald Giant were grown in the field. Immature fruits of Albino are white, whereas those of Emerald Giant are green; mature fruits of both varieties are red. Spinach was purchased locally.

Isolation and Analysis of Chromoplast Proteins

Plastids were isolated by isopycnic sedimentation in silicasol gradients (4). The chromoplasts were subfractionated substantially as described for chloroplasts by Peter and Thornber (11) (*cf.* also ref. 2). Chromoplast membranes were obtained by lysing the chromoplasts in a buffer of low ionic strength and, where indicated, extracted with NaBr to remove peripheral proteins. Membranes were resuspended in extraction buffer to a concentration of 1.1 mg protein per mL as estimated by the bicinchoninic acid method (15). The conditions

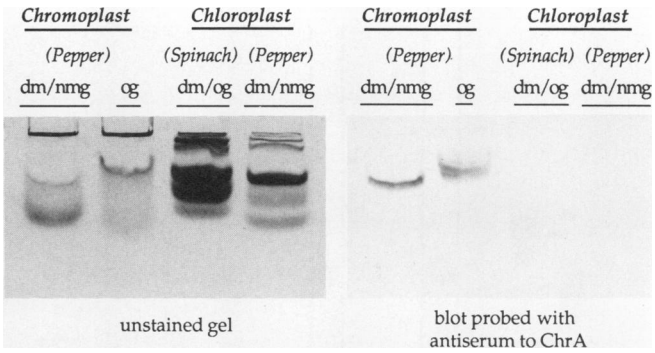

Figure 1. Electrophoresis of chromoplast membrane proteins of *C. annuum* var Albino under nondenaturing conditions. Membranes of pepper chromoplasts were extracted with NaBr and solubilized with 6:3:1:1 dodecyl-β-D-maltoside:nonanoyl-*N*-methylglucamide:SDS:protein (dm/nmg) or 9:1:1 octyl-β-D-glucoside:SDS:protein (og). Equal amounts of protein (30 μg) were used for the chromoplast samples. For comparison, 38 μg of protein from membranes of spinach chloroplasts were extracted with 6:3:1:1.3 dodecyl-β-D-maltoside:octyl-β-D-glucoside:SDS:protein (dm/og) and 30 μg of protein from membranes of chloroplasts of immature fruits of *C. annuum* var Emerald Giant were extracted with 6:3:1:1 dodecyl-β-D-maltoside:nonanoyl-*N*-methylglucamide:SDS:protein (dm/nmg) and run on the same gel. The detergent combinations were selected from among those found optimal for the several preparations. Electrophoresis was continued for 40 min by which time the most rapidly migrating bands had moved about 2.5 cm. Left: photograph of unstained gel. The chromoplast samples resolved into two orange bands whose mobilities depended on the conditions of extraction. The chloroplast samples migrated as multiple, closely spaced green bands. Right: Western blot of gel probed with antibody to ChrA. The more slowly migrating band of the chromoplast samples reacts strongly with antiserum to ChrA.

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[5] Abbreviations: ChrA and ChrB, chromoplast-specific proteins of 58- and 35-kD, respectively; CPC, carotenoid-protein complex.

of nondenaturing and two-dimensional electrophoresis were as described by Peter and Thornber (11).

Authentic ChrA was purified from chromoplasts as described by Hadjeb *et al.* (4).

Antibodies and Immunoblots

Antibodies to ChrA were obtained as described previously (10). Proteins were transferred from gels to Immobilon blotting membrane (Millipore; Bedford, MA) substantially as described by Towbin *et al.* (16) with modifications (17, 18). Blots were treated according to Huynh *et al.* (6).

RESULTS

Our objective was to determine if the chromoplast-specific proteins, ChrA and ChrB, might be associated with the accumulation of carotenoids in ripening fruits of the sweet pepper. We tested this possibility by subjecting chromoplast proteins to two-dimensional gel electrophoresis followed by Western blotting with antibodies to ChrA.

Chromoplasts were isolated from mature, red fruits of *C. annuum* var Albino and solubilized in various combinations of alkyl-glycoside detergents, as recommended by Peter and Thornber (11) for the resolution of thylakoid components. After 40 min of electrophoresis under these nondenaturing conditions, two bright orange bands were resolved (Fig. 1, left). The abundance of the slower migrating band, designated CPC, varied with the conditions of extraction. No discrete carotenoid-containing bands were seen in lanes containing extracts of thylakoid membranes either from leaves of spinach or from immature fruits of *C. annuum* var Emerald Giant. A second set of lanes of the gel shown in Figure 1, left, was blotted and probed with antibodies to ChrA. Antiserum to ChrA reacted strongly with CPC under all conditions of extraction (Fig. 1, right).

After longer intervals of electrophoresis, CPC continued to migrate as a single, compact band, but the more rapidly migrating orange component became progressively more diffuse (data not shown). Blots of 90-min gels still showed CPC as the major reactant with antiserum to ChrA, but faint bands corresponding to lower mobilities were also visible. With certain combinations of detergents, the carotenoid migrates as a single, rapidly migrating zone of free pigment and is not associated with ChrA.

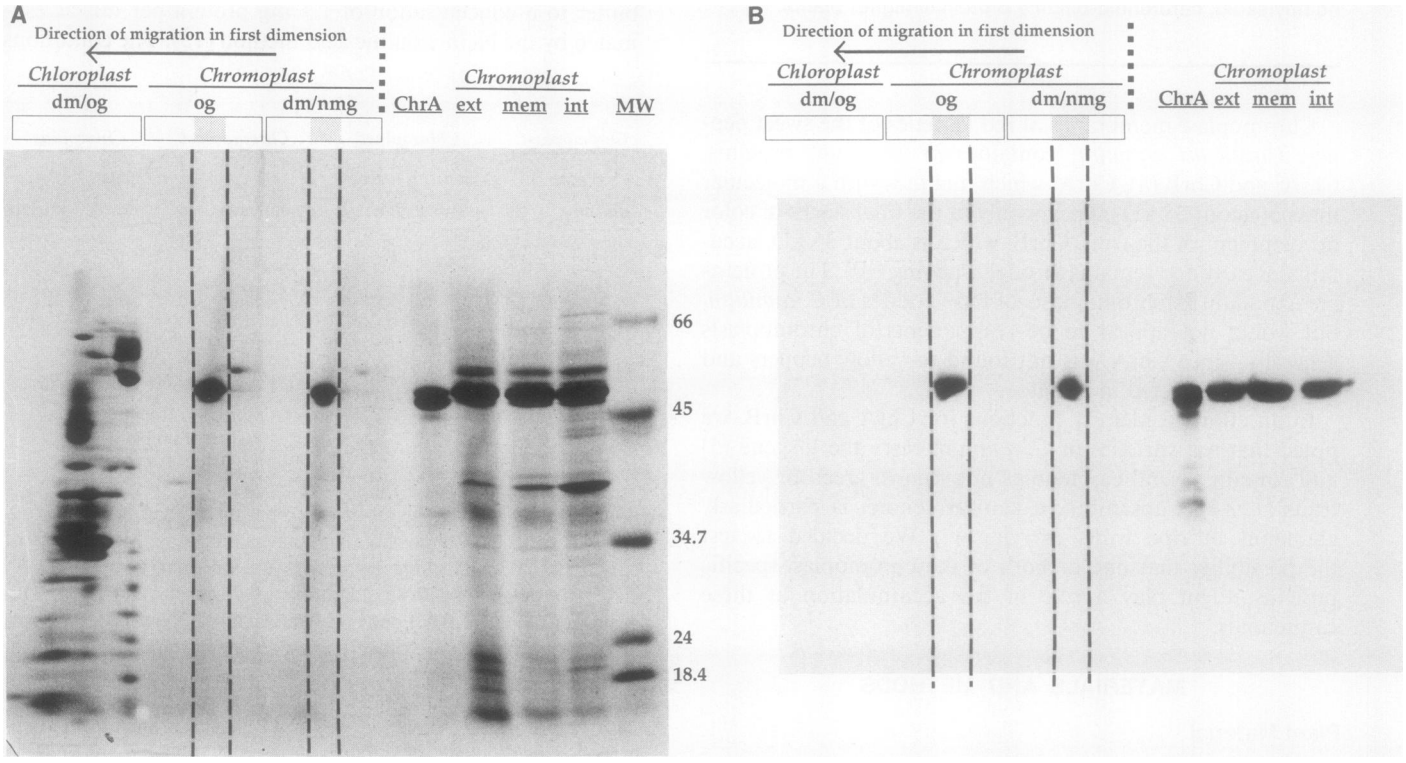

Figure 2. Two-dimensional electrophoresis of chromoplast membrane proteins. Lanes containing extracts of chromoplast and chloroplast membranes from the nondenaturing electrophoresis of Figure 1, left, were sliced longitudinally, denatured with SDS, and placed transverse to the electric field on an SDS-polyacrylamide gel. The slices are represented by rectangles with the positions of the CPC shown as shaded areas. The direction of migration in the first dimension is shown by an arrow; the symbols for these samples are the same as in Figure 1. Additional samples were authentic ChrA, chromoplast membranes extracted with NaBr (ext), native chromoplast membranes (mem), intact chromoplasts (int), and mol wt markers (MW) labeled in 1000s. Left: gel stained with Coomassie blue. Chloroplast membranes resolve into numerous polypeptides aligned under the positions of the various Chl-protein complexes, as shown by Peter and Thornber (11). Chromoplast membranes resolve into one principal protein of about 58 kD and a relatively small number of minor components. The 58-kD protein and several of the minor proteins are aligned with the position of CPC. No proteins run consistently with the more rapidly moving orange band. Right: Western blot of gel stained with antibody to ChrA. Strong signals were given by the principal component of CPC and by each of the chromoplast preparations.

Longitudinal slices of several lanes from similar nondenaturing gels were cut out, denatured in SDS, and subjected to electrophoresis through an SDS-polyacrylamide gradient gel. To provide comparisons with the longitudinal slices from nondenaturing electrophoresis, samples of total chromoplasts, whole chromoplast membranes, NaBr-extracted chromoplast membranes, and purified ChrA were run in parallel lanes. When stained with Coomassie blue (Fig. 2A), CPC is seen to contain a major protein of about 58 kD, which co-migrates with authentic ChrA. Three to five additional components of 20 to 55 kD migrate in a line with CPC. No proteins are consistently associated with the more rapidly migrating orange band. Additional minor components of 15 to 60 kD appear to the right of CPC, corresponding to the region above the complex.

A blot of the SDS gel was also probed with antiserum to ChrA (Fig. 2B). A major signal corresponded to the 58-kD component of CPC. The position of ChrA in the second dimension, as detected by both staining and blotting, always aligned with the position of CPC in the first dimension.

ChrB was detected in one- and two-dimensional gels of whole chromoplast membranes (data not shown), but its association with carotenoids was variable. The behavior of ChrB under different conditions of extraction corresponds to our previous inference that it may be a peripheral protein (4).

DISCUSSION

We can summarize our results as follows: a CPC occurs in chromoplast membranes of *C. annuum* and ChrA is the principal protein component of that complex. The association of ChrA with carotenoids and the lower mobility of the complex compared to that of the free pigment leads us to infer that ChrA is a carotenoid-binding protein.

Carotenoids are associated with the Chl-protein complexes of thylakoid membranes of higher plants (*cf.* refs. 8 and 12), dinoflagellates (13), cyanobacteria (9), and nonoxygenic photosynthetic bacteria (7), but no other CPC has been identified in plants. A soluble CPC has been isolated from cyanobacteria (3) and a number of kinds of CPC are known in animal systems (*e.g.* rhodopsin).

Many additional questions remain to be answered: we do not know which carotenoid or carotenoids are associated with ChrA; we do not know if ChrB or some of the minor proteins may be specifically associated with the carotenoid-ChrA complex; and we do not know if the occurrence of this specific complex correlates with fruit color in pepper or with specific genotypes. It is evident, however, that the association of carotenoids with one or more specific proteins provides new opportunities for the analysis of chromoplast development and of fruit ripening.

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