High-contrast imaging of chlorophyll-containing protein complexes separated by native polyacrylamide gel electrophoresis

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Introduction

Electrophoregrams with bands of green chlorophyll-containing protein complexes separated by native polyacrylamide gel electrophoresis (PAGE) are usually photographed in white light or scanned by a narrow beam of monochromatic light using densitometers. Present CCD cameras allow taking densitograms of a whole gel surface with many samples. However, commercial CCD imaging systems use mostly white or broad-band light scanning for densitometry measurements and are not constructed for the measurements by monochromatic light. In this work we present an application of a home-made two-dimensional CCD imaging system for monochromatic densitometry of chlorophyll-containing proteins separated by PAGE.

Materials and methods

Plant material

*Hordeum vulgare* L. (cv. Akcent) was grown in a growth chamber at 20 °C on the artificial soil composed of perlite and Knop solution. The light regime was 8 h dark / 16 h light (90 µmol m⁻² s⁻¹ of PAR). The detached primary leaf segments of 8-9 days old barley were used for measurements.

Non-denaturing Deriphat-PAGE

Our procedure for non-denaturing Deriphat–PAGE was a modified version of the procedures of Peter and Thornber (1991a, b) and Peter et al. (1991c).

Thylakoid membranes were isolated from unheated or linearly heated (up to 60 and 75 °C, heating rate: 2 °C min⁻¹) barley leaves. Membrane solubilization was carried out using 20% decyl maltoside as a surfactant. Polyacrylamide gel of 8.5% (33.5% (mass/vol) acrylamide, 0.3% (mass/vol) bisacrylamide) was used. For 3 mm thick gel a volume of 13 µl of extract with 14.3 µg of Chl was loaded per line. The gel was electrophoresed at 50 V constant voltage for 15 min and then at 90 V constant voltage for 135 min at 4 °C.

CCD imaging of PPCs separated by non-denaturating PAGE

The imaging system consists of the halogen lamp (150 W), condenser, interference filter (650 or 670 nm, bandwidth 10 nm), beam integrator, pinhole, collimating lens, disc diffuser,
objective and a cheap grayscale CCD camera OS-45D (Oscar, Taiwan) with switched off gama correction. The plane gel with several electroforeograms was placed between the objective and diffuser. Video signal from the camera was grabbed and processed with the Aver-EZCapture video card (AverMedia, Taiwan) plugged into a PC computer. Each pixel of grayscale image of the whole gel has the value from 0 (black) to 255 (white). Spectral sensitivity of the whole CCD imaging system was approximately the same for 650 and 670 nm. Linearity of the CCD response was verified. Mathematical operations with images were performed using the software Matlab 6 (Mathworks, U.S.A.).

Results and discussion

Figure 1 shows electrophoreograms with separated PPCs from barley thylakoids using non-denaturating PAGE scanned with our home-made CCD camera system (see Materials and methods). The first three images in Figure 1 are electrophoreograms scanned at white and monochromatic lights (670 nm and 650 nm, corresponding to red absorption maximum of chlorophyll (Chl) $a$ and $b$, respectively). Due to a higher Chl $a$ content in the separated PPCs the image with the highest contrast was obtained using 670 nm light. As it can be expected the polychromatic scanning of chlorophyll-containing PPCs significantly reduces the contrast of electrophoreogram images. The PSII core complex (CCII) was in this case almost invisible (Fig. 1). Identification of individual bands in the electrophoreogram was performed by measurements of their room temperature absorption and 77K fluorescence emission and excitation spectra. This attribution agrees with results of Peter and Thornber (1991a, b). Densitograms of the first three images in Figure 1 are shown in Figure 2. These were calculated by averaging of gray levels of pixels in each image row and converted to absorbance units by a logarithmic transformation, as raw gray levels of image pixels correspond to gel transmittance and the transmitted light was detected. Again, the highest contrast of separated PPCs is evident for 670 nm light.

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**Fig. 1.** Electrophoreograms of chlorophyll-containing proteins from barley thylakoid membranes separated by PAGE and scanned using white light and monochromatic lights at 670 nm and 650 nm. PSI – photosystem I, CCII(II) – core PSI(II), LHCt – LHC trimer, LHCm – LHC monomer, FP – free pigments. The electrophoreogram right was calculated as a ratio of electrophoreograms scanned at 650 nm and that scanned at 670 nm (for details see text).
As PPCs differ in Chl a/b ratio we aimed to visualize them selectively by a construction of an artificial image comparing images obtained using monochromatic 670 and 650 nm lights. Firstly, we transformed these images to absorbance scale. Than we divided the gray level of each pixel in the 650 nm image by that of the 670 nm image and this ratio multiplied by 255 (range of grayscale levels). The result of these operations is shown in the fourth column in Figure 1 denoted as “ratio”. The lighter bands represent PPCs with higher absorbance ratio A650/A670 indicating their lower Chl a/b ratio. As it can be expected the bands containing LHCs and mainly LHCII complexes with low Chl a/b ratio (LHC trimers – LHCt) are much lighter than the other bands. Mainly PSI a PSII core complexes (CCI and CCII) lacking Chl b are clearly visible as dark bands. Thus, this type of imaging allows fast rough attribution of the electrophoreogram bands to known PPCs. In this sense, for example, we can conclude that the LHCm band represents not only separated PPCs of LHCt band as LHCt band is much lighter than the LHCm band; the LHCm band thus contains in addition the PPCs with higher chl a/b ratio. Further, the band visible between the LHCt and LHCm bands mainly at 670 nm light (see Fig. 1 and also Fig. 2) represents PPC(s) with low Chl b content as in this type of imaging the band is very dark. We also used this “ratio” type of imaging for rough estimation of origin of a new band appearing in electrophoreograms of separated PPCs from thylakoid membranes of preheated barley leaves up to 60 and 75 °C. This new band appeared in the position of CII complex but it is much more intensive than CCII at 670 nm light (Fig. 3, left). In the “ratio” type of imaging this band is very dark (Fig. 3, right). Thus, we can conclude that this new band represents probably the inner antenna photosystem complex without Chl b.

More detail spectral measurements showed that this band really includes antennae of PSI core (not shown).
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References


Fig. 3. Electrophoreograms of chlorophyll-containing proteins from thylakoid membranes of control (25 °C) (see also Fig. 1) and preheated barley leaves separated by PAGE and scanned at 670 nm (left). Terminal temperatures of linear heating (from room temperature, heating rate 2 °C min⁻¹) are indicated. The electrophoreograms in the right were calculated as a ratio of electrophoreogram scanned at 650 nm and that scanned at 670 nm (for details see text).