THE ULTRASTRUCTURE OF PROTEIN AND LIPIDS IN RAPESEED AND X-RAY MICROANALYSES OF TOXIC SEED COMPOUNDS

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Introduction

During the last five years rapeseed has become very important as a source of protein for mankind (ALTSCHUL, 1970). At the Institute of Physiological Botany at the University of Uppsala in Sweden we have analysed Brassica seeds in the transmission electron microscope (HOFSTEN, 1970) and also studied the structure of the rapeseed products in the scanning electron microscope (HOFSTEN, 1974). Chemical analyses show that the seeds contain about 40 % oil, 25 % protein, 25 % polysaccharides and up to 8 % toxic compounds. These compounds are mainly thioglucosides and derivates of phytic acid, and are known to contain sulphur, calcium, magnesium and phosphorus. By means of a new electron microscopic technique called X-ray microanalysis, it is possible to localize these trace elements in thin sections of rapeseed (HOFSTEN, 1973). Both scanning electron microscopy and transmission electron microscopy in combination with X-ray microanalysis have been used. We have analyzed four different Cruciferae species, Brassica napus, Brassica campestris, Sinapis alba and Crambe abyssinica and compared the results with the poppy Papaver somniferum.

Results

Ultrastructure of rapeseed

The seeds of oilseed rape and turnip rape contain two cotyledons and a central part called hypocotyle. It has been known for many years that rapeseeds contain storage protein in special organelles called protein bodies or aleuron grains (RUTKOWSKI, 1971). To observe the localization of the protein bodies in the seed we have worked out a special fixation method for the transmission electron microscopy. At first the peeled seeds are prefixed in 2.5 % glutaraldehyde for 24 hours at 4° C. After rinsing in buffer the material is postfixed in 2 % $KMnO_4$ for 1 hour at +18° C, dehydrated in ethanol and embedded in the plastic Epon. To increase the contrast of the protein in the cells, the thin sections are poststained with lead and uranylacetate.

Figure 1 A shows the protein bodies (PB) in cotyledon cells of Brassica campestris, turnip rape. The cells have a diameter of 10-15 µm and the number of protein bodies vary from one to six. Each cell is surrounded by a thin cell wall (CW), about 500 nm in diameter, and intercellular channels (I) are observed, which are considered to be important for translocation of substances within the seed. The lipids (L) appear as electron-

Figure 1 A: Transmission electron micrograph of cotyledon cells in seeds of turnip rape (Brassica campestris). Protein bodies (PB), lipids (L) and cell walls (CW).

Figure 1 B: Hypocotyle cells in seed of oilseed rape (Brassica napus) rich in protein (PB) and lipids (L). A nucleus (N) is also observed.

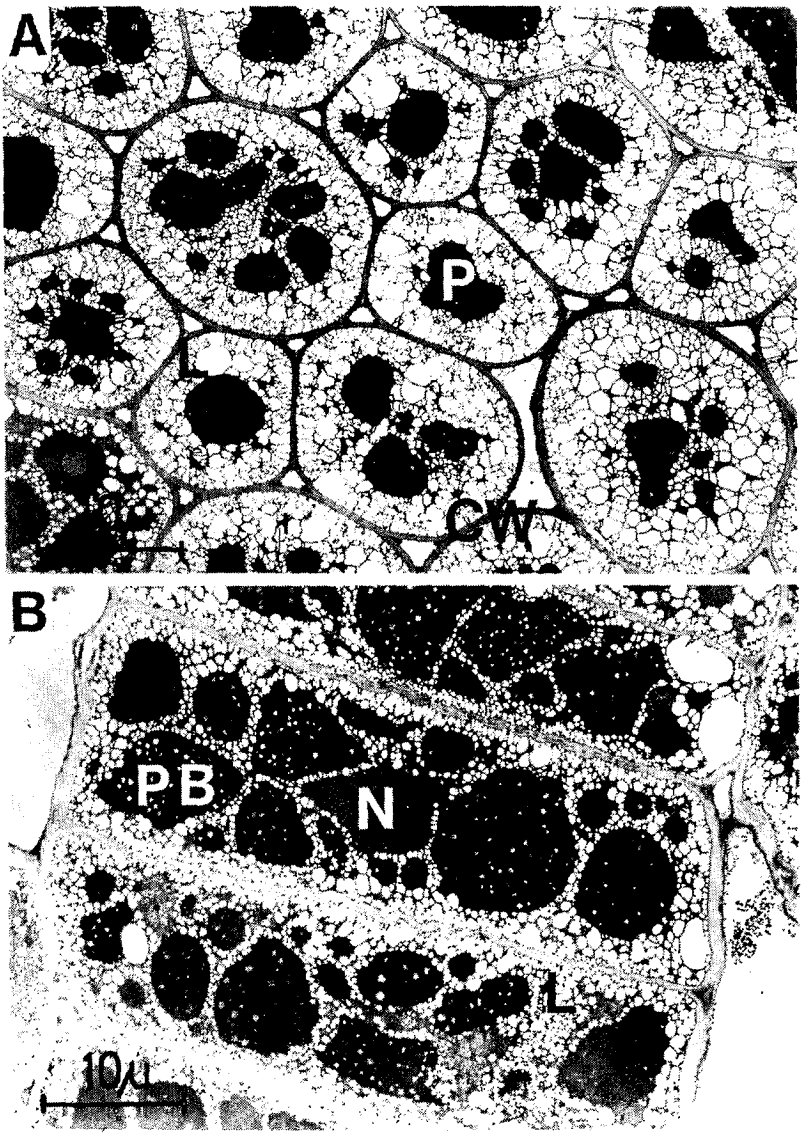


Figure 2 A: A large elongated nucleus (N) with two nucleoli (Nu) in a hypocotyle cell of a tetraploid strain of mustard (Sinapis alba).

Figure 2 B: Protein bodies (PB) with globoids (G) and lipids (L) in a cell of diploid Sinapis alba. Membranous structures (M) and the cell wall (CW) are also observed.

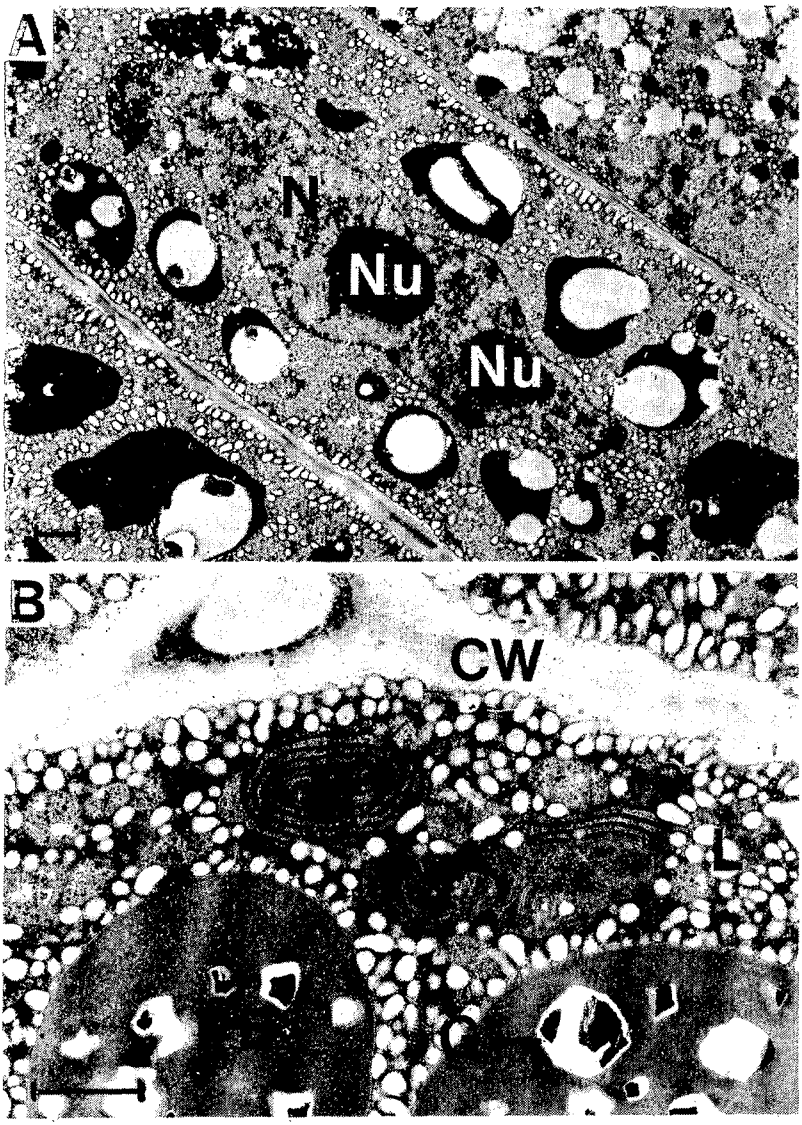
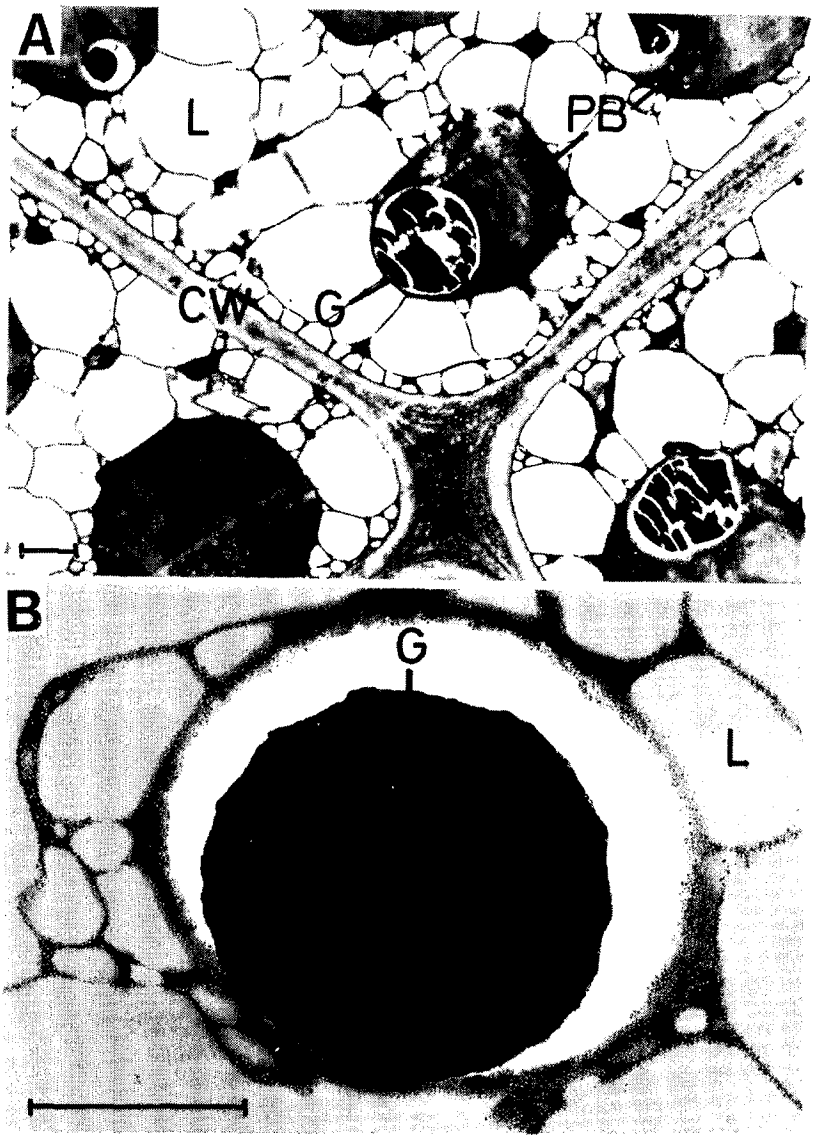


Figure 3 A: Protein bodies (PB) with globoids (G) and lipids (L) in seeds of the poppy, Papaver somniferum.

Figure 3 B: A globoid (G) in a cell of Papaver somniferum at high magnification (40 000 x).



transparent droplets of irregular shape. The protein bodies are conspicuous in the cells of the cotyledon. Up to 35 bodies have been determined in Sinapis alba. Fig. 1B shows two elongated hypocotyle cells of Brassica napus, oilseed rape, where the electron-dense protein bodies stand out clearly against the electron-transparent lipid droplets. In a tetraploid strain of Sinapis alba we have observed a large nucleus with two nucleoli (Fig. 2A). In addition a rich developed membrane system is occasionally observed. The protein bodies contain very electron-dense globoid structures, which sometimes can fall out or shrink during preparation of the material for electron microscopy (Fig. 2B). In Papaver somniferum these globoids are very prominent and have been examined by X-ray analysis to localize trace elements in the seed.

X-ray microanalysis of toxic compounds

We have used three different analytical methods to localize microelements in rapeseed.

The similarity between the methods is that each element in the periodic table will give a characteristic spectrum of X-ray because of their atomic structure when the cell material is bombarded with electrons in the electron microscope. A detector converts the different X-ray energies to a pulse, and the pulses are counted and sorted according to their height by a multichannel analyzer. A computer reads out of the energy spectrum and provides qualitative and semiquantitative information about the multielemental composition of the specimen.

In our experiments on globoids in Crambe seeds (Fig. 4A) we used a scanning electron microscope JSM-U3 with a single dispersive spectrometer. We tested the X-ray emission along a linear scan for the presence of magnesium signals. Two high peaks were observed when the beam passed two of the globoids (see Fig. 4B). Sulphur was analysed from a spot at the centre of a globoid (see arrow on Fig. 4B) and gave a X-ray spectrum with a very high peak (Fig. 4C). To test different analytical methods, the X-ray image for calcium was analysed on a scanned area (Fig. 4D). The X-ray pattern coincided with the transmitted electron image of the globoids.

As mentioned before the protein bodies in Papaver seeds have very electron dense inclusions. The origin of these globoids is not yet known, but with X-ray microanalysis it has been possible to get some information about their chemical composition. For the analysis we have used a transmission electron microscope equipped with the EDAX analytical system. The results of an experiment are shown on Fig. 5. The upper graph, A, gives the spectrum of X-rays measured on a globoid during a 500 second exposure and the lower graph, B, was obtained from an electron transparent background area during the same time. The relative amounts of different elements in the globoid and the background are summarized below the graphs, in C, and they are expressed as counts per 500 seconds. From the figures it is clear that the globoid structure contain large amounts of

Figure 4: STEM and X-ray analyses with single dispersive spectrometer (SDS) on a section of seed of Crambe abyssinica.
A. Transmitted electron image of a protein body (PB), lipid (L) and starch (S).
B. Linear scan for magnesium X-rays.
C. Spectrum of X-ray from a spot rich in sulphur.
D. X-ray image for calcium on a scanned area.

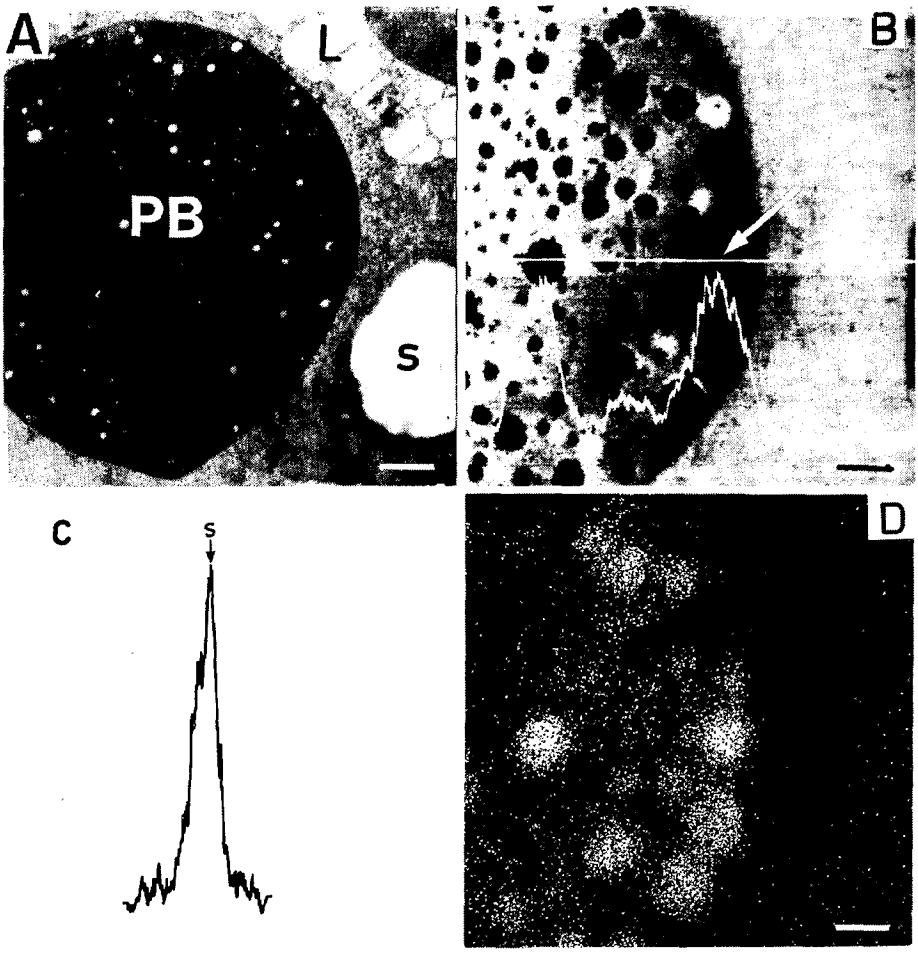
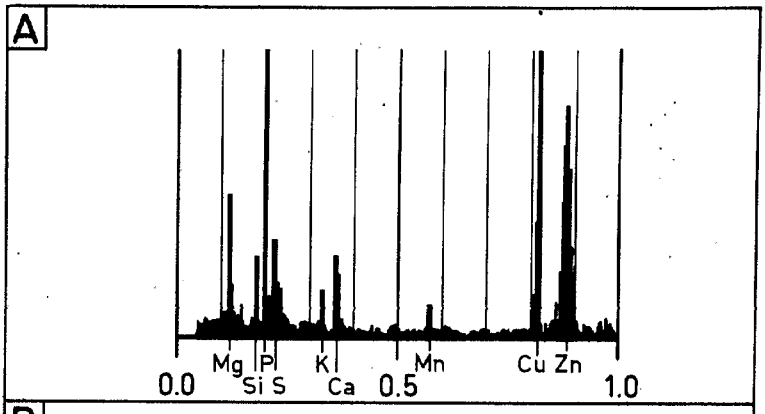


Figure 5: Point analyses of a globoid in a thin section of Papaver seed. The bar graphs were obtained with an EDAX system in a transmission electron microscope and the peaks represent relative amounts of each element.

A. Spectrum for the globoid emission.
B. Spectrum for the background emission.
C. Relative amounts of the analyzed elements as counts per 500 seconds.



C

Element	Mg	Si	P	S	K	Ca	Mn	Cu	Zn	Cl
Globoid	162	99	969	114	54	97	35	>1000	196	
Backgr.		45						>1000	162	45

500 sec. EDAX 50 keV

magnesium, phosphorus, sulphur and calcium. The signals from silica and chlorine probably originate from the plastic embedding material. Large amounts of copper and zinc were found in both graphs and these probably come from the specimen grid. The elements magnesium, calcium and phosphorus may come from the phytic acid, which is very common in seeds. The signal from sulphur can originate from thioglucosides, but to be sure of that, it is necessary to isolate the globoids from the protein bodies and perform an elemental analysis on the isolated material.

Discussion

It is possible to analyse the distribution of protein and lipids in rapeseed by using transmission electron microscopy. By using X-ray microanalyses it is possible to detect such microelements as sulphur, calcium and magnesium within the seed. These elements are strongly bound to the cell material during the electron bombardment. Signals from the specimen grid, fixative chemicals and plastic embedding are difficult to avoid.

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