Abstract

A procedure is described for the rapid (<5 min) isolation of purified, physiologically active chloroplasts from Pisum sativum L. Mitochondrial and microbody contamination is substantially reduced and broken chloroplasts are excluded by washing through a layer containing a treated silica sol. On average the preparations contain 93% intact chloroplasts and show high rates of (14)CO2 fixation and CO2-dependent O2 evolution (over 100 μmol/mg chlorophyll(chl)/h); they are also able to carry out light-driven incorporation of leucine into protein (4 nmol/mg chl/h). The amino-acid contents of chloroplasts prepared from leaves and from leaf protoplasts have been determined. Asparagine is the most abundant amino acid in the pea chloroplast (>240 nmol/mg chl), even thought it is proportionately lower in the chloroplast relative to the rest of the cell. The chloroplasts contain about 20% of many of the amino acids of the cell, but for individual amino acids the percentage in the chloroplast ranges from 8 to 40% of the cell total. Glutamic acid, glutamine and aspartic acid are enriched in the chloroplasts, while asparagine, homoserine and β-(isoxazolin-5-one-2-yl)-alanine are relatively lower. Leakage of amino acids from the chloroplast during preparation or repeated washing was ca. 20%. Some differences exist between the amino-acid composition of chloroplasts isolated from intact leaves and from protoplasts. In particular, γ-aminobutyric acid accumulates to high levels, while homoserine and glutamic acid decrease, during protoplast formation and breakage.