

Abstract

1. Enzyme distribution between chloroplasts and the nonchloroplast parts of green leaf cells of *Spinacia oleracea*, *Nicotiana rustica*, *Vicia faba*, and *Phaseolus vulgaris* have been investigated by use of the nonaqueous chloroplast isolation technique. Whereas pyruvate kinase and peroxidase were located only or mainly outside of the chloroplasts, the other enzymes studied, isocitric dehydrogenase, glutathione reductase, NAD- and NADP-dependent pyridine nucleotide quinone reductase, malic dehydrogenase, NAD- and NADP-dependent glyoxylate reductase, glutamate-oxaloacetate transaminase, NAD-dependent glutamic dehydrogenase, and NADP-dependent aspartic dehydrogenase were both inside and outside of the plastids. In contrast, NADP-dependent glyceraldehyde-3-phosphate dehydrogenase is located only within the chloroplasts.

2. Intact isolated spinach chloroplasts incorporated only a very small amount of labeled carbon from $^{14}\text{CO}_2$ into amino acids in the light. The addition of NH_4Cl did not increase the amount of labeled amino acids and had no effect on the total amount of ^{14}C fixed during short time photosynthesis. However, NH_4^+ caused changes in the pathway of carbon during photosynthesis. In the presence of NH_4^+ , more ^{14}C was incorporated into sugar monophosphates and phosphoglyceric acid than in the absence of NH_4^+ .

3. ^{14}C -labeled glycine and serine fed to intact isolated spinach chloroplasts were neither accumulated nor transformed into other compounds, but ^{14}C -labeled glutamic acid was converted into glutamine. This transformation took place only in the light in chloroplasts containing an intact outer envelope. The addition of NH_4^+ and certain substrates and cofactors did not increase the rate of transformation.

4. The penetration of some amino acids and substrates through the outer envelope of the chloroplasts was investigated on aqueously isolated spinach plastids. It was found that α -ketoglutarate, oxaloacetate, pyruvate, aspartate, and alanine are able to penetrate the envelope although at least for some of these compounds the outer membrane of the chloroplasts acts as a partial barrier.

5. From the experiments reported here and in connection with the results published by other investigators it can be concluded that the most common amino acids such as glutamic acid, aspartic acid, alanine, glycine, and serine are able to penetrate through the outer envelope of the chloroplasts and the synthesis of these amino acids can occur in the leaf cells inside as well as outside of the chloroplasts.