Abstract

Intact chloroplasts from young pea leaves were able to incorporate [3H]thymidine into DNA at relatively high rates (50 pmol mg-1 chlorophyll h-1 or more), using light as the sole energy source. The intact plastids were also able to synthesize DNA in darkness, but only if ATP and MgCl2 (MgATP) were both present. The rates of MgATP-driven assimilation in darkness were equal to or greater than light-driven activity. Neither light nor dithiothreitol pretreatments enhanced thymidine incorporation in darkness, suggesting that enzymes of chloroplast DNA (ctDNA) biosynthesis are not regulated via a thioredoxin-type system. Although exogenous nucleosides (other than [3H]thymidine) were not an absolute requirement, dramatically elevated rates of incorporation (over 300 pmol mg-1 chlorophyll h-1) were seen when adenosine, cytidine, guanosine and thymidine were supplied in combination (500 mmol m-3 each). Radiolabelled DNA synthesized by the isolated chloroplasts was prepared using a new heat extraction method. After digestion by restriction endonucleases, ctDNA synthesized in organello was found to give typical autoradiography patterns for chloroplast DNA. Exonuclease III studies suggested that 5% to 15% of the newly synthesized DNA might be in a closed circular form. MgATP-driven synthesis in darkness was highly age-dependent. Chloroplasts from young (6 to 8-d-old) plants, or alternatively the youngest leaves of more mature plants, were 4—10 times more active than those from older tissues. Although these data do not establish conclusively that replication-type synthesis was occurring in the isolated chloroplasts, they are consistent with this suggestion.