ABSTRACT
Candidate cDNAs for xylem sap proteins (XSPs) were isolated by screening of a cucumber cDNA library prepared from poly[A]+RNA of cucumber roots with an antiserum raised against the XSPs of cucumber. The patterns of expression of corresponding transcripts and the sequences of the cDNAs were examined. Two cDNAs with a glycine-rich domain in each deduced amino acid sequence and whose transcripts were expressed predominantly in roots were selected for further analysis. Both cDNAs encoded proteins with a putative signal sequence, with similar non-glycine-rich domains at the amino terminal of the encoded polypeptides. The corresponding mRNAs accumulated concurrently with the formation of adventitious roots from cuttings of cucumber hypocotyls and the development of vascular tissues, and strong expression of the mRNAs was detected in the root-hair zone of tap roots. These data suggest that the production of the two novel glycine-rich proteins, CRGRP (cucumber root-specific glycine-rich protein)-1 and -2, which are putative XSPs, might be associated with the functions of the vascular tissues in roots. The RNA gel blot analysis with the CRGRP-1- and -2-specific probes revealed that the CRGRP genes expressed only in root but not at all in aboveground organs. When the localization of these mRNAs were examined by in situ hybridization, CRGRP mRNAs were found only in the parenchyma cells in the central cylinder of young lateral roots and it was most abundant in the cells that surrounded xylem vessels in the root-hair zone of the tap root.

In immunoblotting of xylem sap collected from cucumber stem with an antiserum raised against CRGRP-1 that had been produced in an E. coli expression system, the antibodies, which did not cross-react with GRP1.8 of kidney bean, reacted with two proteins, whose mobilities
corresponded to those of proteins deduced from the $CRGRP-1$ and $-2$ cDNAs. Immunohistochemical staining revealed that the CRGRPs accumulated specifically in the lignified walls of metaxylem vessels in the root, stem and leaf and in the lignified cell walls of perivascular fibers in cucumber stems. Immunostaining was also detected in the walls of metaxylem vessels and in the cell walls of adjacent sclerenchyma in the hypocotyl of kidney bean. These data clearly indicate that the novel glycine-rich proteins were produced in the vascular tissue of the root, transported systemically over a long distance via the xylem sap and immobilized in the walls of metaxylem vessels and sclerechyma cells in aboveground organs.