Mechanism of Cyanide Inhibition of Phloem Translocation

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ROBERT GIAQUINTA
Department of Central Research and Development, Experimental Station, E. I. du Pont de Nemours & Co., Wilmington, Delaware 19898
DONALD R. GEIGER
Department of Biology, University of Dayton, Dayton, Ohio 45469

ABSTRACT

Petiolar application of potassium cyanide inhibited 14C-assimilate translocation without affecting source leaf photosynthesis or phloem loading of sucrose in Phaseolus vulgaris. The inhibition of transport was correlated with disruption of the structural integrity of the sieve tubes (sieve pore blockage) rather than impairment of a metabolic process in the translocation path driving translocation.

In light of the above studies and because the inhibition of translocation by path chilling can be attributed to sieve pore blockage and not directly to an inhibition of metabolism, it was of interest to investigate the mechanism of inhibition of transport by cyanide in relation to sieve tube cytology. In this study, the inhibition of translocation by petiolar application of cyanide is correlated with the structure of the sieve tubes rapidly frozen in situ and processed for freeze substitution electron microscopy.

MATERIALS AND METHODS

Two- to 3-week-old bean plants (Phaseolus vulgaris, L. cv. Black Valentine) were trimmed to a simplified source-path-sink system, and the steady-state translocation rate of 14C-assimilates was measured by techniques described previously (6). Prior to the translocation experiments, the surrounding petiole tissue was carefully dissected away leaving a 1- to 2-cm long intact vascular strand (≤1 mm in thickness) (9). Phosphate buffer (50 mM, pH 7.2) or KCN (50 mM phosphate buffer, readjusted to pH 7.2) was applied to the exposed petiolar vascular strand by affixing a polyethylene trough to the dissected region. After KCN treatment, rapid freezing of the vascular strand in situ and processing of the tissue by freeze substitution electron microscopy were as previously described (9). In other experiments, source leaf photosynthesis (8), 14C-sucrose uptake (8), and tissue autoradiography of phloem loading (2) were measured prior to and after a 60-min petiole treatment with 100 mM KCN.

RESULTS AND DISCUSSION

Translocation of 14C-assimilates through an intact vascular strand was monitored by measuring the 14C accumulation rate in the terminal leaflet of the first trifoliate in bean plants (5, 9). After a control rate of translocation was established, the buffer solution surrounding the exposed vascular strand was replaced with either 1, 10, or 100 mM KCN. Cyanide at 1 mM and 10 mM had no effect on translocation, whereas 100 mM cyanide caused a marked inhibition of translocation which was virtually complete after 60 min (Table I). At this point, the vascular strand

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<th>Table I</th>
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<td>Effects of Petiolar Cyanide Treatment on Translocation, Photosynthesis and Sucrose Accumulation in Bean</td>
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<td>Rates were determined after a 60 min petiole treatment with 100 mM KCN. Control rates were determined on the plant prior to cyanide application. Sucrose concentration was 1 mM and the uptake period 30 min. The rate of translocation in the control (with a dissected petiole) was approximately 5 µg C1/min-mm².</td>
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<tr>
<td>Relative Translocation Rate</td>
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<td>Control</td>
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<td>KCN-Treated</td>
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2 To whom reprint requests should be sent.
was rapidly frozen in situ with an isopentane-methyl-cyclohexane mixture cooled by liquid nitrogen (about −170°C) and then processed for freeze substitution electron microscopy (3, 9). To determine whether the observed inhibition of translocation was due to inhibition of source-leaf metabolism by redistribution of the cyanide, several parameters were measured in leaves with petioles being treated with 100 mM cyanide. Table 1 shows that the rates of source leaf photosynthesis and sucrose uptake were not affected by a 60-min cyanide treatment of the path. Moreover, autoradiography of KCN-treated source leaf tissue exposed to 1 mM 14C-sucrose (Fig. 1, A and legend) showed that phloem loading was also not affected. These results indicate that the site of inhibition of translocation by cyanide is at the sieve tube level in the petiole region.

The structural correlates of cyanide inhibition of translocation are shown in Figure 1, B and C. The structure of functioning sieve tubes prepared by freeze substitution electron microscopy is shown in Figure 1B. As previously shown (3, 4, 9), this technique eliminates most of the shortcomings inherent with chemical fixation of sieve tubes and offers the best representation of the structure of functioning sieve tubes. Note that the sieve tube lumen and sieve plate pores are free from cytoplasmic occlusion and that cytoplasmic materials line the lateral membranes of the sieve tubes. This structure agrees well with that reported previously (3, 4, 9). In cyanide-treated petiole tissue, the sieve pores are occluded by cytoplasmic material which appears to be derived from the material that formerly lined the lateral walls (Fig. 1C). In the treated tissue, the lateral walls are relatively devoid of this material which characterized the controls. These results suggest that cyanide causes sieve pore blockage and structural damage to the sieve tubes similar to the damage observed in cold-treated bean petioles (9). This indicates that cyanide inhibition of translocation probably occurs by disruption of the cytoplasm lining the sieve tube, causing this material to be swept into the sieve plate. The inordinately high concentration of cyanide needed to inhibit translocation (100 mM in this study and 500 mM in the study of Ho and Mortimer [10]) also argues against a subtle metabolic inhibition. The mechanism of disruption is not known, but cyanide is highly reactive toward, and forms stable complexes with, a variety of metalloproteins (18). Cyanide may also cause loss of selective permeability of the sieve tube plasmalemma causing sudden pressure release and loss of structural integrity of the sieve tube contents. In any case, cyanide inhibition, like chilling damage, probably occurs by physically altering the structure of sieve tubes and not by directly impairing metabolic reactions necessary for driving translocation. A further likeness between cyanide and chilling inhibition of translocation is the similarity in recovery time after the treatment is removed. Translocation resumes within 90 min after warming a cold-treated bean petiole (5, 7) and recovery seems to depend on re-establishing the structural integrity of the conducting channels by partial reversal and displacement of the cytoplasmic material in the pores (7). These recovery kinetics are similar to the 90-min lag in resumption of translocation reported by Willenbrink (17) when cyanide is removed from the petiole. The lag in resumption of translocation can be viewed as the time required to re-establish the pathways for transport. The results of this study support the possibility that other chemical inhibitors postulated as inhibiting metabolism (10–13) may also disrupt the structural integrity of the sieve tubes. Thus, the results from cyanide inhibition studies, like the chilling studies (1, 9) can be reconciled with the mass-flow theory of translocation.

LITERATURE CITED


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