Aspartate Transcarbamylase Activity in Healthy and Virus-infected Cowpea and Soybean Leaves

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ABSTRACT

The effect of virus infection on aspartate transcarbamylase (ATCase) activity in green tissues was studied using inoculated primary leaves of cowpea and soybean. ATCase activities increased nearly 4-fold in cowpea infected with cowpea mosaic virus (CPMV) and in soybean infected with tobacco ringspot virus (TRSV), as compared to buffer-rubbed controls. Total RNA increases following infection were 5.6- and 1.6-fold that of healthy cowpea and soybean, respectively. Neither virus caused significant change in total buffer-soluble protein. Virus concentrations, as assayed by infectivity, were maximal 8-9 days postinoculation in both hosts.

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Additional key words: cowpea mosaic virus, tobacco ringspot virus.

Aspartate transcarbamylase (EC 2.1.3.2) catalyzes the first reaction unique to pyrimidine biosynthesis (9). Aspartate transcarbamylase (ATCase) activity in healthy etiolated soybean hypocotyls is 5- to 9-fold that in healthy etiolated cowpea hypocotyls (6). We have observed increased activity of ATCase in etiolated cowpea hypocotyls treated with 2,4-D (2) or infected with CPMV, but little increase in the activity of this enzyme in etiolated soybean hypocotyls infected with TRSV or CPMV (6). Using ATCase as an indicator enzyme, we (6) suggested that etiolated cowpea hypocotyls provide pyrimidine nucleotides for virus-induced RNA synthesis via de novo nucleotide synthesis, at least during early infection. In contrast, etiolated soybean hypocotyls might possess the enzymatic capability of synthesizing de novo the pyrimidine requirements for changes in RNA metabolism following virus infection; or alternatively, predominantly utilize a "salvage" pathway to meet these requirements.

This paper establishes that the stimulated ATCase activity following virus infection is not unique to etiolated cowpea tissues, since green leaves of both cowpea and soybean show comparable post-infection increases. Apparent differences between various tissue-virus combinations in the mode of enzyme regulation during later stages of the infection cycle are indicated. Portions of these results have been published in abstract (4).

MATERIALS AND METHODS.—*Viruses*.—The Sb strain of CPMV described by Agrawal (1) and the NC-72 strain of TRSV, provided by G. V. Gooding, were used. CPMV was purified according to Semancik and Bancroft (10). The relative amounts of the electrophoretic forms of CPMV were determined by the method of Niblett and Semancik (7).

Plant materials.—Cowpea, *Vigna unguiculata* (L.) Walp. 'Early Ramshorn', and soybean, *Glycine max* (L.) Merr. 'Wayne', were planted in 15.2-cm (6-inch) diameter pots containing sterilized soil. The plants were grown in growth chambers with 16-hour photoperiods with an average light intensity of 22,000 lux at leaf height, 29 C, and 80% relative humidity. Only primary leaves were used. Trifoliate buds were excised as they formed. Plants were inoculated with virus by rubbing primary leaves dusted with Carborundum with a suspension made by grinding virus-infected leaves with twice their weight of 0.02 M potassium phosphate buffer pH 7.0 (KPO_4). Control plants were dusted with Carborundum and rubbed with 0.02 M KPO_4 only. At intervals postinoculation (PI), primary leaves from 10 plants were harvested, weighed, and stored in sealed plastic bags at −18 C until extracted. Freezing the leaves for short periods of time did not alter the amount of extractable ATCase activity.

Leaf extraction.—Frozen leaves were homogenized for 15 seconds in cold (3 C) 0.1 M ethanolamine-HCl buffer (EA-HCl), pH 10.0, at a ratio of 3 ml/gram fresh weight, at the 5 setting on a Willems Polytron Model PT 20 ST sonicator. The homogenate was centrifuged at 37,000 *g* and 3 C for 15 minutes. Aliquots of the supernatant fluid were frozen for subsequent determinations of buffer-soluble protein and RNA. An aliquot was dialyzed in 0.02 M KPO_4, pH 7.0, for virus assay. The remainder was dialyzed extensively in 0.1 M EA-HCl pH 10.0 (for cowpea) or pH 9.5 (for soybean) for ATCase assays.

Enzyme assay.—Prior to assay, low molecular weight compounds which interfere with the ATCase assay were removed by chromatography through a Sephadex G-75 column (1.5×7.0 cm). Two milliliters of the dialyzed leaf extract were added to the Sephadex G-75 column, which was equilibrated with 0.1 M EA-HCl, pH 10.0 or pH 9.5, for cowpea or soybean, respectively. The first 2-ml fraction eluted after the void volume contained about 85% of the ATCase activity eluted from the column. ATCase activity was assayed as previously described (2), by measuring the carbamyl phosphate (CAP)-dependent incorporation of ^{14}C-aspartic acid into ureidosuccinate (US). Radioactive substrate and product were separated on Dowex-50 columns (H^+ form), and radioactivity in an aliquot of the effluent was measured with a Beckman LS-230 liquid scintillation spectrometer. Enzyme reaction products were identified by thin-layer chromatography (2). The ATCase assay is very reproducible. The coefficient of variation between data from similar experiments ranged from 0 to 13.9%, and averaged 4.5%.

Protein, RNA, and virus assays.—Buffer-soluble protein was determined by the method of Lowry et al. (5). Buffer-soluble RNA was determined by the method of

Key and Shannon (3). Virus concentration was determined by local-lesion assay after leaf extracts were dialyzed and frozen: CPMV was assayed on pinto bean, *Phaseolus vulgaris* L. 'Pinto', and TRSV on cowpea. Eight half-leaves were inoculated per treatment. Average number of local lesions per half-leaf was corrected for dilution to express virus concentration as number of local lesions per gram fresh weight. Data presented are representative of experiments done at least twice in duplicate.

RESULTS.—ATCase activity in healthy cowpea leaves declined through 3 days PI, and remained constant through 12 days PI. In CPMV-infected leaves, ATCase activity paralleled that in healthy tissues through 6 days PI, although generally running somewhat higher. After 6 days PI, ATCase activity increased sharply in CPMV-infected leaves until, at 7-8 days PI, it was nearly 4.0-fold greater than that of healthy cowpea. It remained high through day 12 (Fig. 1). CPMV concentration, as determined by infectivity, peaked at 9 days PI (Fig. 1). RNA also increased rapidly in CPMV-infected leaves. By

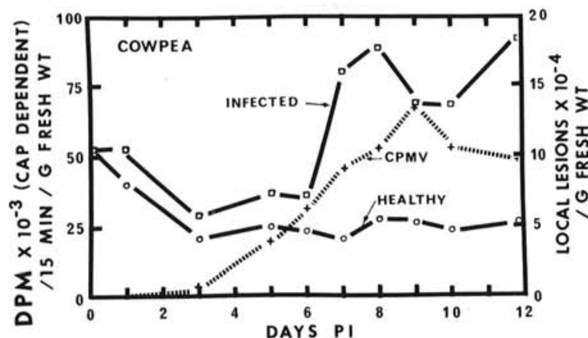

Fig. 1. Aspartate transcarbamylase activity and cowpea mosaic virus (CPMV) concentration in healthy and CPMV-infected cowpea leaves. Enzyme activity is expressed as carbamyl phosphate-dependent disintegrations per minute per gram fresh weight during a 15-minute reaction. The ^{14}C-ureidosuccinate formed is separated from the ^{14}C-aspartate substrate by elution from a Dowex-50 column. CPMV concentration is expressed as number of local lesions per gram fresh weight as assayed on pinto bean.

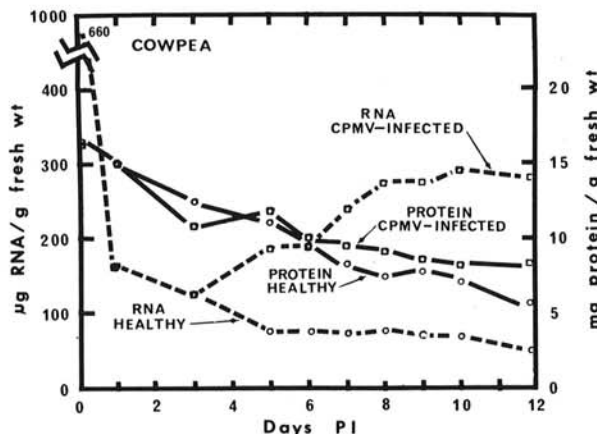

Fig. 2. Buffer-soluble RNA and buffer-soluble protein from healthy cowpea leaves and cowpea leaves infected with cowpea mosaic virus (CPMV). Data are from the experiment shown in Fig. 1.

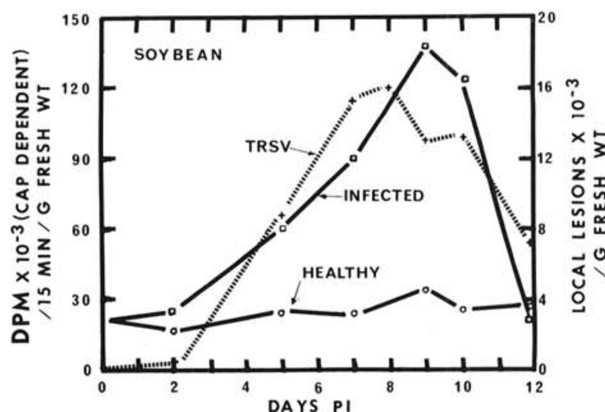

Fig. 3. Aspartate transcarbamylase activity and tobacco ringspot virus (TRSV) concentration in healthy and TRSV-infected soybean leaves. Enzyme activity is expressed as carbamyl phosphate-dependent disintegrations per minute per gram fresh weight during a 15-minute reaction. The ^{14}C-ureidosuccinate formed is separated from the ^{14}C-aspartate substrate by elution from a Dowex-50 column. TRSV concentration is expressed as number of local lesions per gram fresh weight as assayed on cowpea.

12 days PI, it was 5.6-fold greater in CPMV-infected leaves than in healthy leaves (Fig. 2). Buffer-soluble protein (Fig. 2) in both healthy and CPMV-infected leaves declined steadily, and showed little quantitative difference.

CPMV exists in slow- (S) and fast-migrating (F) electrophoretic forms (7, 8). Specific infectivity of the F-form is approximately twice that of the S-form (7). Under our experimental conditions, CPMV purified from cowpea leaves 3 days PI possessed a F/S ratio of 0.09, and at 10 days PI, a ratio of 1.00, which indicates that the relative CPMV concentration in leaf extracts is less than the infectivity assay indicates during later stages of infection.

In soybean, ATCase activity in healthy leaves remained constant over the period analyzed (Fig. 3). ATCase activity in TRSV-infected leaves increased progressively and was 4-fold greater than the activity in healthy leaves by 9 days PI. ATCase activity declined rapidly between 10 and 12 days PI. TRSV concentration was maximum 8 days PI, and then declined. Buffer-soluble RNA in TRSV-infected leaves was 1.6-fold greater than in healthy plants at 9 days PI. Buffer-soluble protein showed little change (Fig. 4).

The major radioactive product of ATCase assays of the leaf extracts from healthy and virus-infected cowpea and soybean was ureidosuccinate (Fig. 5). Little or no aspartate; or dihydroorotate (the next intermediate in the pyrimidine pathway) was detected in the eluates of the reaction mixture from the Dowex-50 columns. The identity of the small peak of radioactivity at the solvent front is unknown, but its appearance was not totally CAP-dependent.

DISCUSSION.—ATCase activity increased approximately 4-fold in cowpea and soybean leaves after infection with CPMV and TRSV, respectively (Fig. 1, 3), and nearly 6-fold in etiolated cowpea hypocotyls following CPMV infection (6). Assuming ATCase activity to be a valid indicator, then de novo pyrimidine

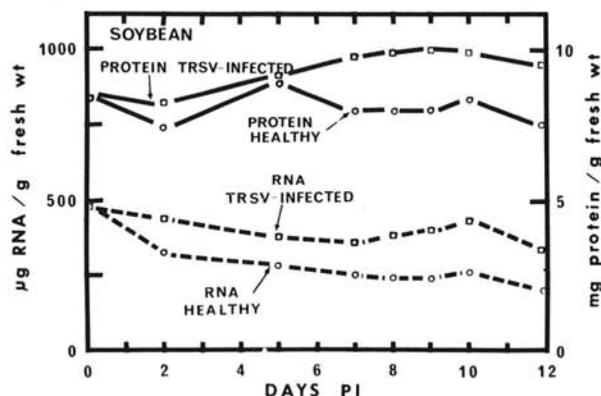

Fig. 4. Buffer-soluble RNA and buffer-soluble protein from healthy soybean leaves and soybean leaves infected with tobacco ringspot virus (TRSV). Data are from the experiment shown in Fig. 3.

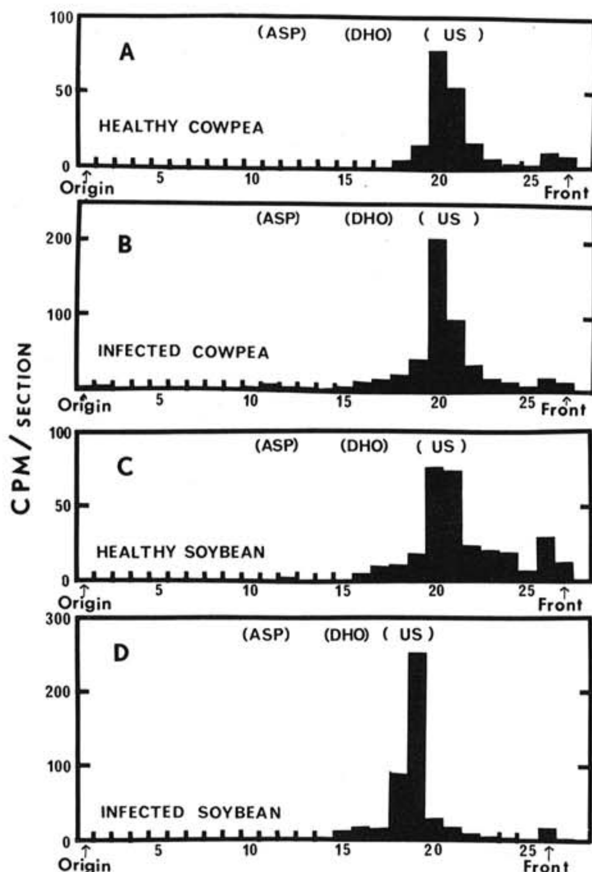

Fig. 5-(A to D). Distribution of radioactivity in thin-layer chromatograms of the concentrated reaction products of a 15-minute assay of comparable healthy and 8-day-infected leaves. **A**) healthy cowpea leaves, **B**) cowpea mosaic virus-infected cowpea leaves, **C**) healthy soybean leaves, **D**) tobacco ringspot virus-infected soybean leaves. Standards of aspartate (ASP), dihydroorotate (DHO), and ureidosuccinate (US), determined colorimetrically (2), are indicated. Developing solvent was isopropanol-HCl-water (65:16.6:18.4, v/v) used with cellulose chromatogram sheets (Eastman 6604). Each section represents 5 mm.

synthesis is stimulated in all three systems after infection. This seems reasonable, since total buffer-soluble RNA also increased in all three systems.

In contrast, ATCase activity showed little increase in CPMV- or TRSV-infected etiolated soybean hypocotyls, although the latter showed considerable virus synthesis (up to 225 μg/g fresh weight) (6). In comparison, RNA content and endogenous ATCase activity were up to 2- and 9-fold higher, respectively, in soybean than in cowpea. Until changes in total RNA and RNA synthesis following TRSV infection in soybean hypocotyls are known, speculation on the source of pyrimidines is more difficult. ATCase activities may change little because endogenous ATCase levels are adequate, because a pyrimidine "salvage" pathway is being utilized, or because pyrimidines are being mobilized to the infected tissues from other organs.

In inoculated primary leaves of both CPMV-infected cowpea and TRSV-infected soybean, virus concentration peaked at 8-9 days PI and then declined. In contrast, only in the latter case did ATCase activity drop markedly during this time. Neither system showed a marked drop in buffer-soluble RNA. This contrasts with etiolated cowpea hypocotyls infected with CPMV, where virus concentration increased throughout the 9-day period analyzed, while both ATCase activity and buffer-soluble RNA declined by the seventh day after inoculation (6). These observations may reflect differences in the pathways used to provide nucleotides during later stages of viral infection. The mechanisms whereby ATCase activity is "turned on" and "turned off" during virus infection are of interest, and are currently being investigated.

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