an electron intensity peak along interatomic axes describes qualitatively the observed photoelectron normal emission intensity. Indeed, the Cu 3*p* photoelectron ADP at 56.6 eV is well described by a comprehensive multiple-scattering, spherical-wave simulation of the electron distribution (*10*). Similarly, the Auger electron ADP at 59 eV (Fig. 2E) shows an angular distribution nearly identical to the 56.6-eV image (Fig. 2D): the physical origin of the dip is not extraordinarily sensitive to electron energy.

What causes the dip in intensity along normal emission in the $M_{2,3}M_{4,5}M_{4,5}$ Cu Auger electron ADPs and—from the measurements of Frank *et al.* (*2*)—along interatomic axes in general? Any physical model must account for all electron angular distribution measurements from crystalline samples. To summarize, the interatomic axis, electron intensity dips are:

1) Observed in several different materials: Cu $M_{2,3}M_{4,5}M_{4,5}$ [(*11*) and this work], Pt *CVV* (*2*), and Ni $M_{2,3}M_{4,5}M_{4,5}$ (*11*).

2) Observed for Auger transitions considered to be "quasi-atomic" based upon their energies and linewidths (*12, 13*).

3) Not observed in all materials nor all Auger transitions in materials where it is observed: Al *LVV* (*11*) and Cu *LVV* (*14*).

4) Not observed in photoemission under identical conditions where they are observed in Auger emission (this work).

5) Not observed for atoms on top of surfaces (*2, 11*).

6) Not predicted by quantum-scattering models using *s*-wave continuum waves (*15*).

7) Predicted by quantum-scattering models in which higher (l = 3) angular momentum continuum waves are used (*16*).

Our work here eliminates any explanation based upon an electron-scattering effect alone. Note, however, that some (*16*) scattering models do predict the interatomic "dips" seen in Auger electron emission, even if these models are too complex to provide a clear physical explanation. It seems that the diffraction initial state (Auger final state neglecting scattering) must play a role: either this initial state is not atomic, involving coherent emission from several atoms because of the participation of valence levels in the Auger relaxation transition, or this initial state is atomic but some mechanism exists for destructive interference between its angular momentum components.

The material and transition dependence of the phenomenon can be explained if it only occurs for Auger transitions involving *d* orbital angular momentum levels or (more generally) partly delocalized valence electron levels that may participate in chemical bonds. The "quasi-atomic" nature of the Auger transitions and the partial success of theories containing only single-center emission at predicting the dips argue against a valence-level involvement, but allow the possibility that some strong, inherent emission anisotropy exists in the Auger electron intensity. Note that even if the Auger transition populates all magnetic sublevels equally in an atomic-like continuum wave and hence has isotropic emission intensity, each sublevel certainly has anisotropic phase.

What are the implications of our results for the use of Auger electrons for determining the structure of surfaces? The dips observed by Frank *et al.* are real: simple extensions of the scattering models successful for photoelectrons but which ignore the Auger transition details will not be useful for analyzing Auger angular distributions from some systems. However, these dips are not observed for all Auger transitions nor even all Auger transitions in the same material. The classical "shadowing" model used by Frank *et al.* cannot be used even as a simple heuristic, because it attributes the electron intensity attenuation, or dips, along interatomic axes that they observe to a scattering effect. Through our Auger electron and photoelectron comparison, we have shown that scattering effects are not responsible for the observed differences. Until the physical origin of this phenomenon is understood in detail and its sensitivity to nonstructural variables in the emission process checked, structures proposed solely from the analysis of Auger electron ADPs—such as the extraordinary atop adsorption of Ag atoms on Ag monolayers on Pt reported by Frank *et al.* (*17*)—should be independently verified.

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Evidence for an Inter-Organismic Heme Biosynthetic Pathway in Symbiotic Soybean Root Nodules

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The successful symbiosis of soybean with *Bradyrhizobium japonicum* depends on their complex interactions, culminating in the development and maintenance of root nodules. A *B. japonicum* mutant defective in heme synthesis in culture was able to produce heme as a result of its symbiotic association with the soybean host. The bacterial mutant was incapable of synthesizing the committed heme precursor δ-aminolevulinic acid (ALA), but nodule plant cells formed ALA from glutamate. In addition, exogenous ALA was taken up by isolated nodule bacteria of the parent strain and of the mutant. It is proposed that bacterial heme found in nodules can be synthesized from plant ALA, hence segments of a single metabolic pathway are spatially separated into two organisms.

BRADYRHIZOBIUM JAPONICUM IS THE bacterial endosymbiont of soybean (*Glycine max*) that functions as a nitrogen-fixing organelle within cells of a plant organ called a root nodule. It has been suggested that the heme prosthetic group of leghemoglobin, a plant protein abundant in nodules, is a bacterial product (*1*), but the

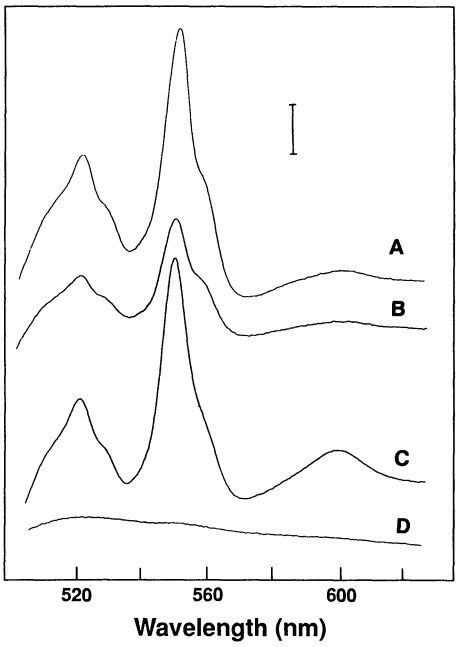

Fig. 1. Absorption spectra of extracts of symbiotic and cultured cells of strains I-110 and MLG1. (**A**) I-110 bacteroids (protein, 3.5 mg/ml); (**B**) MLG1 bacteroids (protein, 3.5 mg/ml); (**C**) I-110 cultured cells (protein, 6.8 mg/ml); (**D**) MLG1 cultured cells (protein, 6.8 mg/ml). The vertical bar represents a change in absorbance of 0.01. The difference spectra that resulted from subtraction of ferricyanide-oxidized from dithionite-reduced spectra were generated as described (*16*). Cultured cells were grown in a yeast extract–glycerol medium, harvested in the log phase of growth, and disrupted with a French Press to obtain cell extracts (*16*). Bacteroids were isolated from nodules harvested after 40 days of plant growth (*16*). Such preparations are devoid of plant mitochondria as judged by the carbon monoxide–insensitive cytochrome c oxidase activity. The lack of ALA synthase activity in strain MLG1 bacteroids (Tables 1 and 2) shows that this mutant did not revert to wild type during symbiotic growth.

coordination of rhizobial heme and plant hemoglobin syntheses is unknown. However, soybean root nodules that are incited by a bacterial mutant defective in the enzyme δ-aminolevulinic acid (ALA) synthase, which is one in the pathway of heme synthesis, fix nitrogen and contain leghemoglobin (*2*): thus the exact role of *B. japonicum* in the formation of the heme moiety of the soybean hemoglobin remains unclear. We now attempt to clarify the respective roles of the plant and bacterial partners in nodule heme synthesis.

Because plant hemoglobin is successfully synthesized in soybean nodules incited by a *B. japonicum* ALA synthase mutant (*2, 3*), a question arises as to whether that enzyme is required for the expression of bacterial heme and hemoproteins in symbiotic cells (bacteroids). ALA is the first of seven committed precursors in the heme biosynthetic pathway, and it is formed solely from glycine and succinyl–coenzyme A by ALA synthase in free-living *B. japonicum* cells grown in culture (*2, 4*). The normal growth and respiration of *B. japonicum*–cultured cells defective in heme (*4*) show that the status of bacterial heme in nodules incited by the ALA synthase mutant must be determined directly, and cannot be assumed from the gross phenotype of those nodules. The *B. japonicum* ALA synthase mutant MLG1 (*2*) was defective in heme when grown in liquid culture as seen by absorption difference spectra (Fig. 1), but when grown symbiotically in nodules the mutant cells expressed significant quantities of mesoheme and protoheme as indicated by the absorption at 552 and 560 nm, respectively (Fig. 1). These bacterial hemes are the prosthetic groups of cytochromes, which are the major hemoproteins in *B. japonicum* and in most other bacteria. The mutant strain MLG1 expressed approximately 50% as much cytochrome heme as was found in bacteroids of the parent strain I-110 (Fig. 1). These observations show that heme was present in strain MLG1 bacteroids despite the lesion in ALA synthase, and that heme expression by the mutant was restored when in symbiosis with the plant.

Although heme is present in the mutant

Table 1. ALA synthase, ALA dehydratase, and PBG deaminase activities in extracts of parent strain I-110 and mutant strain MLG1 cells grown symbiotically or in culture. Extracts of cultured and symbiotic cells were obtained as described in Fig. 1 except that cultured cells were harvested at the stationary phase of growth. ALA-synthase activity was measured at 30°C as previously described (*4*); ALA was purified from the reaction mixture with the use of ion exchange chromatography and quantitated by a colorimetric assay. ALA dehydratase and PBG deaminase activities were monitored at 30°C and 37°C, respectively, as previously described (*13, 14*).

Source	Activity (nmol hour^{-1} mg^{-1})*		
	ALA synthase	ALA dehydratase	PBG deaminase
Cultured cells			
I-110	1.1 ± 0.10	1.2 ± 0.02	0.25 ± 0.01
MLG1	0	1.8 ± 0.15	0.04 ± 0.002
Symbiotic cells			
I-110	1.4 ± 0.01	1.6 ± 0.04	0.19 ± 0.01
MLG1	0	1.2 ± 0.03	0.20 ± 0.01

*Nanomoles of product formed per hour per milligram of protein. Products formed by ALA synthase, ALA dehydratase, and PBG deaminase are ALA, PBG, and uroporphyrin, respectively. The data are presented as the average of at least three measurements ± the standard deviation.

Table 2. ALA synthesis from glutamate or glycine in fractions of nodules incited by strain I-110 or MLG1, and in uninfected roots. ALA synthesis from glutamate (10 μCi, 50 μM final concentration) was assayed at 37°C for 1 hour as described (*4*) in nodule fractions from 40-day-old plants or in extracts of uninfected roots from 23-day-old plants. The data presented are the average of three determinations, and the standard deviations were less than 10%. ALA was extensively purified (*4*) and radioactivity measured in a scintillation counter. ALA was identified by its behavior on an ion exchange column and with various solvents, as well as by paper chromatography as described, where most of the radioactivity comigrated with a single Ehrlich-positive spot indicative of the ALA-pyrrole (formed by the reaction of ALA with ethyl acetoacetate) (*15*). In addition, ALA formed from glutamate was identified by the nonradioactive assay described in Table 1, where we observed that an absorption spectrum of the pyrrole formed from reaction with ethyl acetoacetate was identical to that formed with authentic ALA. The ALA formed from glycine (1 μCi, 50 μM final concentration) was quantitated as described for glutamate-dependent synthesis, except that glutamate was not present in the reaction mixture. ALA synthesis from glycine is a measurement of ALA synthase activity. The observation that ALA formed from glutamate occurred primarily in the plant fractions and glycine-dependent ALA synthesis occurred mostly in the bacterial fraction (of strain I-110) shows that cross-contamination of fractions did not occur to any significant extent.

Fraction	Incorporation of counts into ALA (cpm hour^{-1} mg^{-1} of protein)	
	3,4-[^{3}H]glutamate	2-[^{14}C]glycine
Nodules		
I-110		
Plant cytosol	7062	49
Bacteroids	197	2715
MLG1		
Plant cytosol	1981	66
Bacteriods	0	25
Uninfected roots	810	Not determined

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strain MLG1 bacteroids, it is not clear whether heme synthesis occurs in those cells, or whether the plant host provides the heme moiety for bacterial hemoprotein synthesis. The heme precursor porphobilinogen (PBG) is synthesized directly from ALA, and it is subsequently metabolized by PBG deaminase in the heme synthetic pathway. In addition to being a heme precursor, PBG is also a precursor for the dipyrromethane cofactor of PBG deaminases from animals, plants, and bacteria (*5*). Thus an organism lacking PBG would be deficient in PBG deaminase activity. Cultured cells of *B. japonicum* mutant strain MLG1 contained less PBG-deaminase activity than did the parent strain I-110 (Table 1), which is expected of a strain deficient in the ability to synthesize PBG in situ. However, strain MLG1 bacteroids isolated from soybean root nodules had the same amount of PBG-deaminase activity as the wild type (Table 1), an indication that PBG is available to those cells notwithstanding the mutation in ALA synthase. Two explanations are consistent with our findings: (i) An alternative mode of ALA synthesis occurs in bacteroids that is absent in cultured cells, or (ii) the soybean host can provide the bacteroids with ALA or PBG. Provision of heme or any other heme precursor by the plant would not explain the normal PBG deaminase activity in strain MLG1 bacteroids. Either possibility implies that bacterial enzymes catalyze at least some of the steps in heme synthesis, but the distinction between the two hypotheses can be made by ascertaining which symbiont synthesizes ALA.

The plant fraction of soybean root nodules lacks ALA synthase activity (*6*), but plants form ALA for chlorophyll synthesis from glutamate by the so-called C_5 pathway (*7*), and some evidence shows that non-chlorophyll plant tetrapyrroles are derived from this pathway as well (*8*). In addition, several nonphotosynthetic bacteria synthesize ALA from glutamate (*9*), and thus there are precedents for speculating that either the plant or bacterial symbiont could make ALA without ALA synthase in soybean root nodules. ALA synthesis from ^{3}H-labeled glutamate or from ^{14}C-labeled glycine (ALA synthase activity) was measured in plant and bacteroid fractions of soybean nodules incited by strain I-110 or mutant strain MLG1 (Table 2). Glutamate-dependent ALA synthesis was found in the plant fractions of both nodule types, but it was not present in either of the bacterial fractions. ALA synthase activity, as measured by the incorporation of labeled glycine, was present in strain I-110 bacteroids, but not in strain MLG1 bacteroids or in either of the plant fractions, results that are in agreement with colorimetric assays of ALA synthase activity (*2, 5*) (Table 1). These results show that nodules incited by mutant strain MLG1 contain ALA synthesis activity only in the plant nodule cytosol; we propose that this glutamate-dependent ALA formation is responsible for rescuing mutant strain MLG1 under symbiotic growth conditions. The lower ALA synthesis activity found in the plant cytosol of nodules incited by strain MLG1 as compared to those incited by the wild type may be a consequence of the poorer apparent health of plants inoculated with the mutant bacteria (*3*).

The data do not rule out the possibility that bacteroid heme is synthesized from plant ALA only in nodules elicited by the mutant. However, plant ALA synthesis activity in wild-type nodules was considerably greater than that found in uninfected roots (Table 2), which suggests a symbiotic role for this activity. Conversely, bacterial ALA synthase activity in bacteroids of strain I-110 was not appreciably different from that of cultured cells (Table 1) despite the greater amount of hemoprotein in bacteroids (*10*) (Fig. 1). In addition, exogenous ALA was taken up by isolated bacteroids of parent strain I-110 (Fig. 2), as would be necessary for bacterial heme to be synthesized from plant-derived ALA. The observed rate of ALA uptake was greater than the reported uptake rates of other organic acids that are essential for bacteroid metabolism (*11*). These data suggest that bacteroid heme found in the wild-type parent strain can be synthesized from plant ALA as proposed for the mutant bacteroid, but they do not exclude synthesis of heme in wild-type cells from bacterial ALA as well.

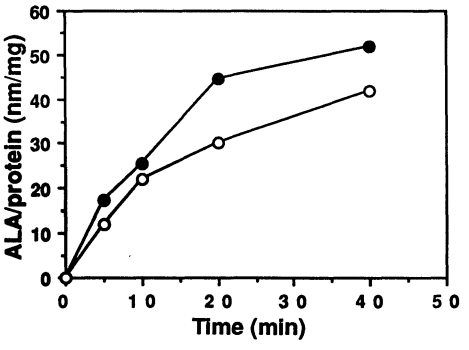

Fig. 2. ALA uptake by bacteroids of strain I-110 and MLG1. Suspensions (1 ml) of bacteroids (2.7 mg and 3.6 mg of protein for I-110 and MLG1 cells, respectively) in 50 mM sodium phosphate buffer, pH 7.5, were incubated at 37°C with vigorous shaking in the presence of 1 μCi of [4-^{14}C]ALA and 1 μmol of total ALA. The reactions were stopped as indicated by dilution with chilled phosphate buffer containing unlabeled ALA (1 mM), followed by rapid centrifugation and one washing in the same buffer. Radioactivity in washed cells was measured in a scintillation counter. Zero-time values were subtracted from all time points, and each time point shown is the average of duplicate samples. Each measurement was within 10% of the average; ●, I-110; ○, MLG1.

We have provided evidence for a heme synthetic pathway in soybean root nodules in which the initial steps are carried out by the plant host and the subsequent steps by the bacterial symbiont. The data explain how plant hemoglobin heme can be a rhizobial product, but not require bacterial ALA synthase. The mechanism of heme formation proposed here for soybean nodules may not extend to other legume symbioses, as other rhizobial *hemA* mutants on their respective legume hosts incite nodules that lack hemoglobin heme (*12*). Synthesis of heme by *B. japonicum* from soybean ALA in nodules provides a potential point for regulation of the endosymbiont by its host.

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