Symbiosis, 21 (1996) 115–127
Balaban, Philadelphia/Rehovot

Metabolic Fate of Energetic Amino Acids in the Aposymbiotic Pea Aphid *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae)

I. LIADOUZE, G. FEBVAY*, J. GUILLAUD, and G. BONNOT
Laboratoire de Biologie Appliquée, INSA, UA INRA 203, Bât. 406, 69621 Villeurbanne Cedex, France, Tel. +33-72438356, Fax. +33-72438511

Received February 27, 1996; Accepted May 22, 1996

Abstract

Amino acid metabolism of aposymbiotic pea aphids obtained by an antibiotic treatment was analysed by the use of ^{14}C-radiolabeled tracers in isotopic equilibrium, and compared with data obtained previously in symbiotic conditions. The interconversions between energetic amino acids were little affected, but the synthesis of three essential amino acids (threonine, isoleucine and lysine) from carbons of common amino acids was interrupted after antibiotic treatment. An accumulation of glutamate from precursors normally converted to these essential amino acids and an increase of glutamate or glutamine in the honeydew plus exuviae of aposymbiotic aphids were likewise observed. These results validate the role of the symbiotic bacteria in the synthesis of essential amino acids.

Keywords: Homoptera, Aphididae, *Acyrthosiphon pisum*, pea aphid, symbiosis, essential amino acids, glutamic acid

*The author to whom correspondence should be sent.

0334-5114/96/$05.50 ©1996 Balaban

1. Introduction

The phloem sap, on which aphids (Homoptera: Aphididae) feed almost exclusively, may be considered as nutritionally poor: it is practically devoid of lipids and its nitrogen content is constituted mainly of free amino acids of unbalanced composition (Raven, 1983; Ziegler, 1975; Rahbé et al., 1990). Aphids display an obligate association with bacterial endosymbiotes of the genus *Buchnera* (Munson et al., 1991), harboured in cells called bacteriocytes and located within the haemocel (Buchner, 1965). It was demonstrated that these symbiotic microorganisms are not responsible for the biosynthesis of the peculiar fatty acids found in aphids (De Renobales et al., 1990; Febvay et al., 1993; Rahbé et al., 1994). On the contrary, a number of studies suggest that these endosymbiotes may be involved in the synthesis of amino acids (Buchner, 1965; Mittler, 1971; D *115*w, direct amino acid synthesis by the symbiotes has been only shown in the case of incorporation of inorganic sulphate into the sulphur amino acids for *Myzus persicae* (Sulzer) (Douglas, 1988), and direct experimental evidence has been provided for the synthesis of tryptophan by the symbiotes of the pea aphid *Acyrthosiphon pisum* (Harris) (Douglas and Prosser, 1992). These biosynthetic capabilities of endosymbiotes were recently confirmed by comprehensive molecular genetic analysis (see Baumann et al., 1995 for review). Nitrogen flow through the pea aphid and its symbiotes was studied by labeling experiments with heavy nitrogen showing that glutamic acid is utilized by the symbiotes as a nitrogen source for numerous amino acids including essential ones (Sasaki et al., 1991; Sasaki and Ishikawa, 1995). Nevertheless, the biosynthesis of the C-skeleton of these amino acids was not taken into consideration in these studies.

Recently, we have demonstrated the synthesis in symbiotic pea aphid of three essential amino acids (threonine, isoleucine and lysine) from carbons of common amino acids (Febvay et al., 1995). To explain the results of amino acid interconversions obtained in this study and in accordance with earlier suggestions (see Douglas, 1989 for review), we proposed a theoretical model of compartmentation involving the biosynthesis of these amino acids by the symbiotic bacteria and the supply of glutamate as a main amino acid transported from the insect to its symbiotes. This central role of glutamate in amino acid metabolism was also supported by data available on the symbiotes of the pea aphid (Whitehead and Douglas, 1993; Sasaki and Ishikawa, 1995) and on analyses of free amino acids in symbiotic and aposymbiotic aphids (Liadouze et al., 1995).

The specific purpose of the present study was to clarify which metabolic transformations were achieved solely by the aphid, among those detected in the whole symbiotic aphid. This comparison then allows to delineate the

specific contribution of the symbiosis to this metabolism. This was achieved by mild treatment with antibiotic in artificial diet to obtain aposymbiotic aphids (Rahbé et al., 1993). The method, based on the use of ^{14}C-radiolabeled tracers in isotopic equilibrium and developed for the analysis of the metabolism of energetic amino acids in symbiotic pea aphid (Febvay et al., 1995), was then carried out on these aposymbiotic aphids.

2. Material and Methods

Insects

A parthenogenetic clone of *A. pisum* (Ap-LL01) was established from a field infestation on lucerne in France (October 1986). The stock of culture was maintained in the laboratory on broad bean seedlings (*Vicia faba* L. var. Aquadulce) in Plexiglas cages (21°C, 70% R.H., L16:D8). As described previously (Febvay et al., 1995), alate viviparous adults, reared at low density on *Vicia* seedlings for 2–3 days, were allowed to lay progeny on young *Vicia* plants. After 6 hours, 15 young larvae were sampled and used for an experiment with an amino acid precursor.

Metabolism analysis

The method used for the metabolic analysis of aphids was precisely described earlier (Febvay et al., 1995). A single neonate larva was deposited in a metabolism cage, which was then sealed with a Parafilm® sachet stretched over the experimental chamber. Artificial diet (20 µl) with a radiotracer was aseptically enclosed in this sachet and made available to the feeding of the larva. In order to study the metabolism of aposymbiotic aphids, this artificial diet was supplemented, during the 4 first days, with rifampicine (50 µg/ml). The efficiency of this treatment to eliminate symbiotic bacteria was already demonstrated (Rahbé et al., 1993). As fully reported previously (Febvay et al., 1995), the first three cages were used at the end of each experiment (7 days) to determine the total activity in the expired CO_2 (trapped in Carbo-Sorb), in the aphid and in its honeydew plus exuviae (digested in 200 µl of Soluene-350). With other cages (5 to 10, depending on the experiment) the aphid was treated separately from its honeydew plus exuviae for amino acid analysis. After hydrolysis under nitrogen in HCl vapours at 110°C during 24 h, these samples were analysed, using the procedure described earlier (Febvay et al., 1995), on an automatic amino acid analyser (Beckman 6300). This analyser was coupled with an on-line radioactivity flow detector (Flo-one/Beta A500), which

allowed for the detection and quantification of radiolabeled compounds (Bonnot and Febvay, 1995).

Artificial diet and radioisotopes used

The composition of the diet used in all experiments was that of the A5 diet (Febvay et al., 1988), with a sucrose content adjusted to 586 mM (20% w/v). Just before the beginning of an experiment, the radiolabeled compound was aseptically added to 800 µl of this artificial diet. Seven independent experiments were realized with the following amounts of radioisotopes added to the diet: 0.7 MBq of L-[U-^{14}C]-alanine (5.62 GBq/mmol), 0.75 MBq of L-[U-^{14}C]-aspartic acid (8.14 GBq/mmol), 0.7 MBq of L-[U-^{14}C]-glutamic acid (9.25 GBq/mmol), 1.0 MBq of L-[U-^{14}C]-glutamine (7.4 GBq/mmol), 1.0 MBq of L-[U-^{14}C]-glycine (3.88 GBq/mmol), 1.0 MBq of L-[U-^{14}C]-proline (9.62 GBq/mmol), or 0.7 MBq of L-[U-^{14}C]-serine (5.5 GBq/mmol). Radioisotopic tracer amounts represented only 0.4-1.6 % of the corresponding amino acid amount present in the diet, and the resulting modification of diet composition could therefore be neglected.

Uptake of diet

We evaluated the volume of food ingested per milligram of aphid fresh weight in a separate experiment (same conditions) using inulin, a non metabolized compound, as described by Wright et al. (1985). 0.35 MBq of inulin-(hydroxy [^{14}C] methyl) (81.4 kBq/mmol) was added to 800 µl of artificial diet and the expired CO_2, the aphid, its honeydew, and its exuviae, after digestion with Soluene-350, were counted separately at the end of the experiment.

3. Results and Discussion

Growth performance and diet uptake

As already reported by several authors (Ohtaka and Ishikawa, 1991; Prosser and Douglas, 1991; Liadouze et al., 1995), aposymbiosis greatly affected aphid performances. This was revealed in our experiments by the significant difference ($P < 0.001$) of the aphid weight at the end of experiment (7 days): 0.62 ± 0.03 mg for the aposymbiotic aphids (mean ± SE, n=75) *vs* 1.21 ± 0.04 mg (n=85) for the symbiotic aphids.

The relationship between the volume of diet ingested during the experimental period and the fresh weight of aposymbiotic aphids exhibited,

as for symbiotic aphids, a good linear regression ($r^2 = 0.85$). The comparison of the slopes of ingested volume *vs* aphid weight between symbiotic and aposymbiotic aphids showed no statistical difference and indicated that the aposymbiosis treatment had no effect on the relative feeding rate of aphids (data not shown). This result was in good accordance with that of Prosser et al. (1992). Indeed, if aposymbiotic aphids fed less than the symbiotic control aphids, these authors showed on artificial diet that this was a consequence of the small size of the treated aphids, and thus that the antibiotic treatment had no specific effect on the ingestion process. More recently, Wilkinson and Douglas (1995a) showed a similar result with symbiotic and aposymbiotic larval aphids reared on plants. As a consequence, a global feeding rate was calculated here for both aposymbiotic and symbiotic aphids:

Volume of diet ingested (µl) = 4.04 × aphid fresh weight (mg) – 0.47 ($r^2 = 0.95$).

From this relation, the volume of diet ingested was used to calculate the ingested radioactivity in experiments performed with radiolabeled amino acids, and to estimate the fraction unaccounted for during the analysis of radioactivity distribution among compartments (CO_2, aphid, honeydew and exuviae). This fraction unaccounted for represented for example, the label incorporated into volatile compounds, other than CO_2, that were not trapped. Nevertheless, the estimation of this fraction by direct difference between *ingested* and *recovered* activities showed that aphid carcass digestion in Soluene was dependent on the size of aphids (correlation coefficient = 0.83, $P < 0.001$) and that this digestion appeared complete for aphids with a fresh weight ≤0.6 mg. This "unaccounted for" fraction was therefore corrected to take into account this systematic bias.

Fate of amino acids

For each amino acid precursor studied, the distribution of radioactivity among the different compartments was compared between aposymbiotic and symbiotic aphids (Fig. 1). After antibiotic treatment, the main noticeable difference appeared when glutamate or its amide (glutamine) were used as labeled precursor. In these cases, an increase of radioactivity recovered in honeydew plus exuviae was noted. As detailed previously (Febvay et al., 1995) and measured in aposymbiotic aphids in some experiments (data not shown here), the radioactivity of this compartment was mainly represented by that of the honeydew: depending on the labeled amino acid studied, the activity recovered in the exuviae (i.e. the remnant cuticles) counted only for 1 to 4% of the total ingested radioactivity and could therefore be neglected.

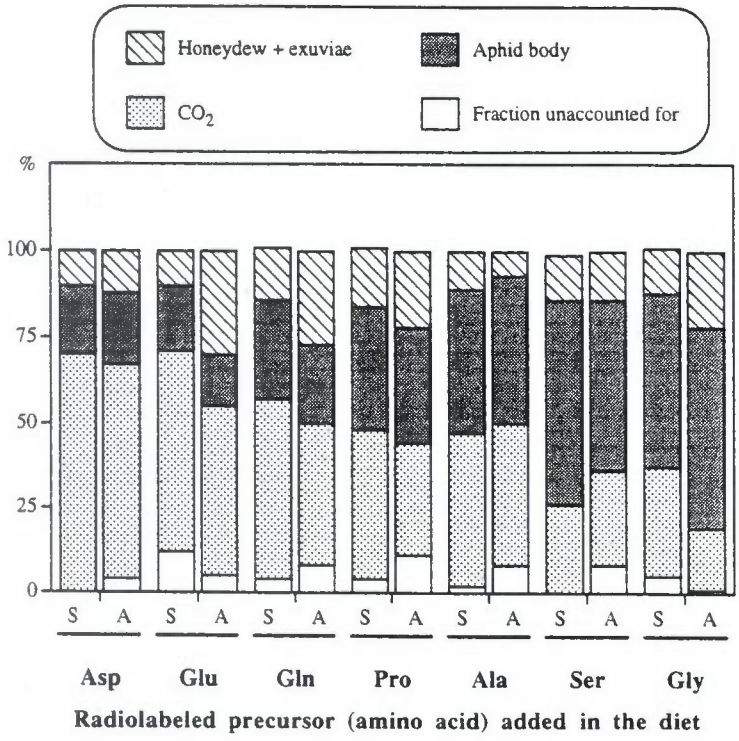

Figure 1. Comparison between symbiotic aphids (S) and aposymbiotic aphids (A) of the distribution among analysed compartments of carbon derived from each dietary ^{14}C-amino acid. Data are expressed in %, means were calculated after angular transformation.

The intensity of radiolabeling recovered in the aphid hydrolysates from each radiotracer is given in Table 1. The quantity (Q_a) of each amino acid (cold + labeled), necessarily incorporated to give the measured radioactivity within body hydrolysates, was therefore computed in nmole per mg fresh weight (Table 1). These values could be used as rescaling factors for each column graph of Fig. 1 in order to express the percentages into quantities of processed precursors. Although feeding similar quantity of diet per weight unit, aposymbiotic aphids incorporated less aspartate, glutamate, glutamine, proline and glycine but more serine than did symbiotic ones. To build the same quantity of living material, but of different composition, the aposymbiotic aphids seemed to use differently the available nutrients as this was shown previously by the much higher concentration of free amino acids in aposymbiotic aphids (Liadouze et al., 1995).

Table 1. Radioactivity recovered (A_a) and quantity of precursor (Q_a) involved by incorporation of this labeling, in hydrolysates of symbiotic and aposymbiotic aphids for each precursor studied.

Precursor	A_a (cpm/mg fresh weight)		Q_a (nmole/mg fresh weight)	
	Symbiotic aphid	Aposymbiotic aphid	Symbiotic aphid	Aposymbiotic aphid
Asp	29220 ± 610 (11)	11870 ± 510 (9)	2.9 ± 0.1	2.1 ± 0.1
Glu	17170 ± 490 (11)	13520 ± 1020 (9)	5.8 ± 0.2	4.8 ± 0.4
Gln	14070 ± 250 (11)	34810 ± 1800 (8)	19.6 ± 0.4	16.2 ± 0.8
Pro	46870 ± 1070 (10)	31280 ± 3310 (7)	13.5 ± 0.3	10.4 ± 1.1
Ala	15660 ± 710 (9)	24150 ± 3980 (5)	10.2 ± 0.5	11.6 ± 1.9
Ser	27650 ± 490 (10)	65280 ± 4670 (5)	16.0 ± 0.3	21.7 ± 1.6
Gly	49740 ± 1340 (7)	33700 ± 1520 (10)	40.1 ± 1.1	23.5 ± 1.1

Values are expressed as means ± SE (number of repeats).
Taking into account the differences in specific radioactivity between experiments, the quantity (Q_a) of each amino acid (cold + labeled) incorporated was computed, in nmole per mg fresh weight, using the formula: $Q_a = (A_a \times Q_d) / A_d$; where A_a represents the radioactivity recovered in the hydrolysates (cpm/mg fresh weight); Q_d, the concentration of amino acid precursor (cold + labeled) in the diet (nmole/ml) and A_d, the specific radioactivity of the diet (cpm/ml).

The proportions of labeling found in the various amino acids after hydrolysis of aphid are shown in Fig. 2. In the aposymbiotic aphids, the rates of metabolic transformations from all the substrates tested into aspartate, glutamate, proline and alanine were very similar to those observed with

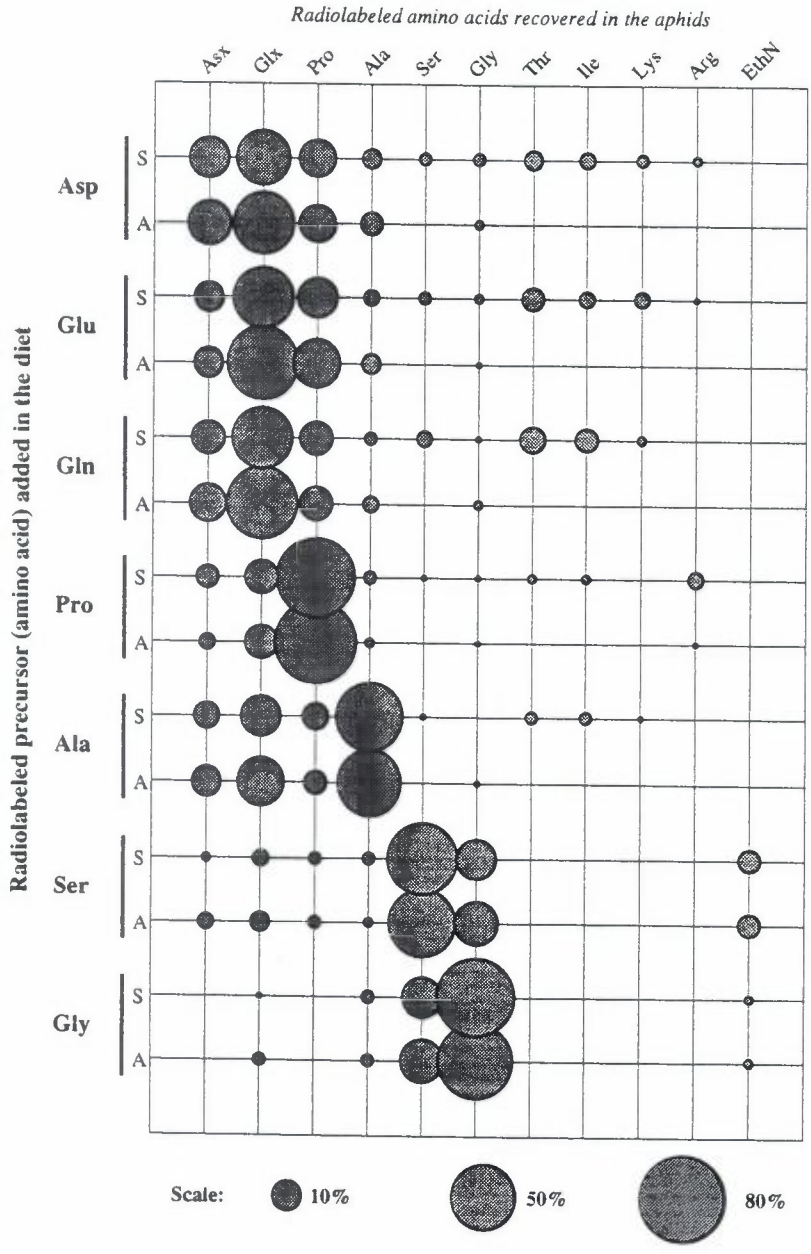

Figure 2. Comparison between symbiotic aphids (S) and aposymbiotic aphids (A) of the distribution of radioactivity recovered in the amino acids of aphid body from each studied dietary ^{14}C-amino acid.

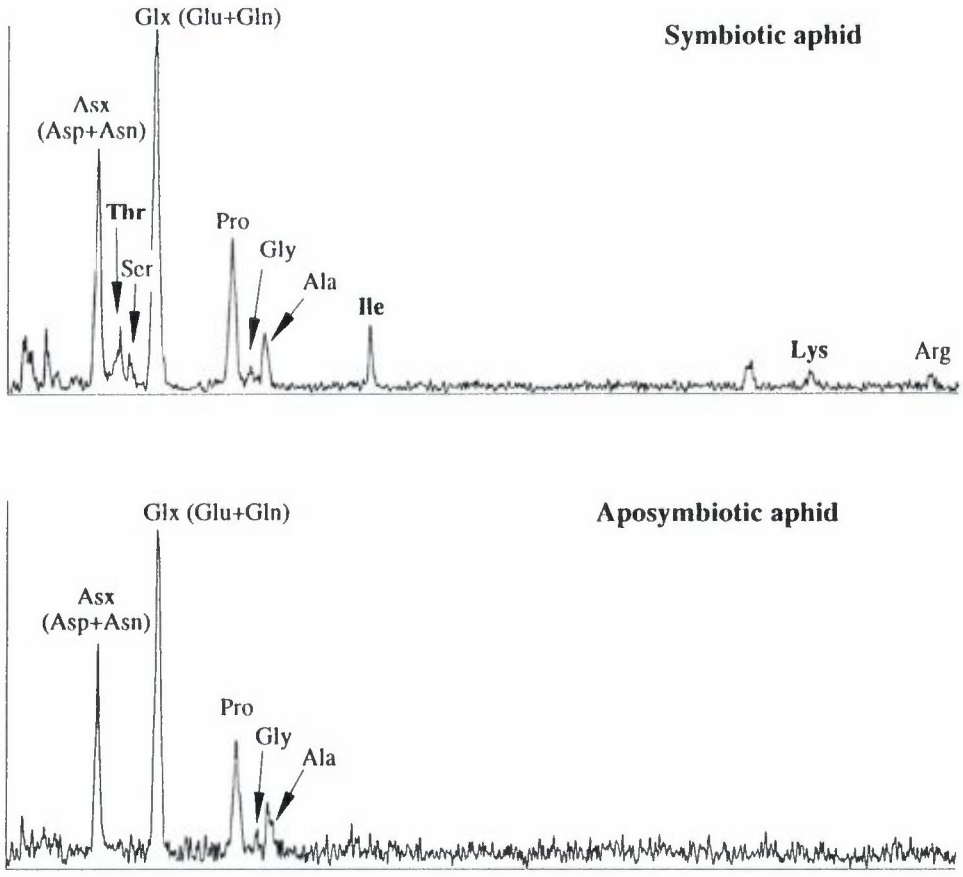

Figure 3. Example of radiochromatograms of individual aphid (symbiotic and aposymbiotic) fed with aspartate as labeled precursor showing the disruption of essential amino acid synthesis in aposymbiotic aphid.

symbiotic aphids. In contrast, if the interconversion between glycine and serine was unaffected by aposymbiosis, the biosynthesis of serine from other sources was reduced and became undetectable. However, the most striking result was that the synthesis of the three essential amino acids (threonine, isoleucine and lysine) from different amino acid precursors, revealed in the symbiotic aphid, was completely interrupted after antibiotic treatment. To illustrate this disruption of synthesis, radiochromatograms of individual symbiotic and aposymbiotic aphids fed with aspartate as labeled precursor are presented in Fig. 3.

As already discussed (Febvay et al., 1995), the incorporation of radioactivity into these three amino acids, was always observed (in symbiotic aphids) when labeled glutamate was present in the aphid body, either produced as an intermediate product or directly assimilated from the diet. The results obtained with aposymbiotic aphids further substantiate the hypothesis of glutamate being a central point in symbiotic exchanges: when the synthesis of essential amino acids was disrupted, the proportion of recovered glutamate was significantly increased (except with proline as precursor): 32.3 ± 3.2% in symbiotic aphid *vs* 42.6 ± 3.9% in aposymbiotic aphid with aspartate as precursor; 42.4 ± 3.5% *vs* 55.5 ± 8.1 % with glutamate; 43.9 ± 2.7% *vs* 56.0 ± 7.3% with glutamine; 19.2 ± 1.5% *vs* 27.1 ± 4.3% with alanine and 13.3 ± 1.0% *vs* 12.6 ± 1.9% with proline.

In the excreted material, the labeling recovered was almost exclusively found in the same chemical form as supplied in the diet, and thus represented mainly the non-assimilation of dietary amino acids. Consequently, the rise of labeling in the excreted material of aposymbiotic aphids with glutamate or glutamine as precursor may be due to a lower utilization of these amino acids, resulting in a decrease of their assimilation by aposymbiotic aphids. Nevertheless, the analysis of amino acids in honeydew plus exuviae after hydrolysis did not allow us to distinguish between glutamate and glutamine. This increase of labeling in the excreted material may also result from the high concentrations of glutamine found in the honeydew of aposymbiotic aphids (Sasaki et al., 1990; Prosser and Douglas, 1991). This glutamine was shown to be generated from glutamate by glutamine synthetase, and interpreted as a detoxification product of ammonia (Wilkinson and Douglas, 1995b).

As procedures to bring the symbiotic bacteria into long-term culture have not yet been achieved, the comparison of metabolism between symbiotic and aposymbiotic aphids is the sole approach to investigate the role of symbiotes in such associations. Whatever the methods used to break the symbiotic association (injection of symbioticidal substances, Ehrhardt, 1966; heat treatment, Ohtaka and Ishikawa, 1991; antibiotic-infused plants, Douglas and Prosser, 1992; antibiotic in artificial diet, Rahbé et al., 1993) the aposymbiotic aphids do not produce any viable progeny and the experiment cannot be conducted on a second untreated generation, which would allow to discard the direct effect of the antibiotic on metabolism. To diminish this problem, the antibiotic treatment done at the beginning of the larval life was time-limited. Therefore, as previously shown (Rahbé et al., 1993), the aphids had a significant period of time to recover from the antibiotic shock. Moreover, the balanced diet used here has been shown to be especially convenient to aposymbiotic aphids (Sasaki et al., 1991).

The purpose of our study was to demonstrate the physiological role of symbiotes in providing amino acids to their host through metabolism. Although this fact is generally accepted without a true direct demonstration because all animals are thought unable to synthesize essential amino acids, the comparison of metabolic capacities in symbiotic and aposymbiotic animal, as achieved here, was necessary to clarify the involvement of symbiosis in metabolism. In this context, and even if the direct inhibition of synthetic activities by antibiotic treatment cannot be definitely excluded, the results obtained here are the first direct evidence of the synthesis of the essential amino acids, threonine, isoleucine and lysine, by the symbiotic bacteria in pea aphid. Moreover, as the participation of symbiotes to host metabolism is not necessarily limited to the provision of essential amino acids, this study allows to estimate clearly which amino acid, and how much of it, is produced by the aphid itself. The amino acid metabolism appeared very active for both symbiotic and aposymbiotic aphids. This observation remains quite surprising in a nutritional context where the amino acid composition of the diet was designed to meet the nutritional needs for anabolism (Febvay et al., 1988), and therefore to limit metabolic conversions.

In aposymbiotic aphids, the increase in glutamate + glutamine labeling from amino acid precursors that are normally converted to these essential amino acids in symbiotic aphids and the higher excretion of glutamate and glutamine in honeydew are consistent with the pivotal role of glutamate in the interconversion between amino acids, as hypothesized in our compartmentation model for amino acid synthesis by the symbiotic aphid (Febvay et al., 1995). While glutamic acid was shown to be a nitrogen donor for the synthesis of essential amino acids by the symbiotes in aphids (Sasaki and Ishikawa, 1995), the present study indicates the important contribution of its C-skeleton to the syntheses of several amino acids (including essential amino acids).

Acknowledgements

We thank G. Duport for her help in insect cultures. We are grateful to Y. Rahbé for helpful discussions and comments on the manuscript. I. Liadouze was supported in part by the Agricultural Academy of France (Dufresnoy grant).

REFERENCES

Baumann, P., Baumann, L., Lai, C.Y., Rouhbakhsh, D., Moran, N.A., and Clark, M.A. 1995. Genetics, physiology and evolutionary relationships of the genus *Buchnera*: Intracellular symbionts of aphids. *Annual Review of Microbiology* **49**: 55–94.

Bonnot, G. and Febvay, G. 1995. Fourier processing of liquid chromatograms using flow radioactive detection. *Analytical Biochemistry* **224**: 354–363.

Buchner, P. 1965. Aphids. In: *Endosymbiosis of Animals with Plant Microorganisms*. Buchner, P., ed., Interscience, New York, pp. 297–332.

De Renobales, M., Cripps, C., and Kinsey, M. 1990. Lipid biosynthesis in adult *Acyrthosiphon pisum*: effect of age and symbiont population. *Archives of Insect Biochemistry and Physiology* **14**: 85–92.

Douglas, A.E. 1988. Sulphate utilization in an aphid symbiosis. *Insect Biochemistry* **18**: 599–605.

Douglas, A.E. 1989. Mycetocyte symbiosis in Insects. *Biological Reviews* **64**: 409–434.

Douglas, A.E. 1990. Nutritional interactions between *Myzus persicae* and its symbionts. In: *Aphid-Plant Genotype Interactions*. Campbell, R.K. and Eikenbary, R.D., eds., Elsevier, Amsterdam, pp. 319–328.

Douglas, A.E. and Prosser, W.A. 1992. Synthesis of the essential amino acid tryptophan in the pea aphid (*Acyrthosiphon pisum*) symbiosis. *Journal of Insect Physiology* **38**: 565–568.

Ehrhardt, P. 1966. Die Wirkung von Lysozyminjektionen auf Aphiden und deren Symbionten. *Zeitschrift für vergleichende Physiologie* **53**: 130–141.

Febvay, G., Bonnot, G., Malosse, C., and Einhorn, J. 1993. A peculiar fatty acid, (Z,Z)-9,12,17-octadecatrienoic acid, identified in the phospholipids of the pea aphid, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae). *Experientia* **49**: 915–918.

Febvay, G., Delobel, B., and Rahbé, Y. 1988. Influence of amino acid balance on the improvement of an artificial diet for a biotype of *Acyrthosiphon pisum* (Homoptera: Aphididae). *Canadian Journal of Zoology* **66**: 2449–2453.

Febvay, G., Liadouze, I., Guillaud, J., and Bonnot, G. 1995. Analysis of energetic amino acid metabolism in *Acyrthosiphon pisum:* a multidimensional approach to amino acid metabolism in aphids. *Archives of Insect Biochemistry and Physiology* **29**: 45–69.

Liadouze, I., Febvay, G., Guillaud, J., and Bonnot, G. 1995. Effect of diet on the free amino acid pools of symbiotic and aposymbiotic pea aphids, *Acyrthosiphon pisum*. *Journal of Insect Physiology* **41**: 33–40.

Mittler, T.E. 1971. Dietary amino acid requirements of the aphid *Myzus persicae* affected by antibiotic uptake. *Journal of Nutrition* **101**: 1023–1028.

Munson, M.A., Baumann, P., and Kinsey, M.G. 1991. *Buchnera* gen. nov. and *Buchnera aphidicola* sp. nov., a taxon consisting of the mycetocyte-associated, primary endosymbionts of aphids. *International Journal of Systematic Bacteriology* **41**: 566–568.

Ohtaka, C. and Ishikawa, H. 1991. Effects of heat treatment on the symbiotic system of an aphid mycetocyte. *Symbiosis* **11**: 19–30.

Prosser, W.A. and Douglas, A.E. 1991. The aposymbiotic aphid: an analysis of chlortetracycline-treated pea aphid, *Acyrthosiphon pisum*. *Journal of Insect Physiology* **37**: 713–719.

Prosser, W.A., Simpson, S.J., and Douglas, A.E. 1992. How an aphid (*Acyrthosiphon pisum*) symbiosis responds to variation in dietary nitrogen. *Journal of Insect Physiology* **38**: 301–307.

Rahbé, Y., Delobel, B., Calatayud, P.A., and Febvay, G. 1990. Phloem sap composition of lupine analyzed by aphid stylectomy: methodology, variations in major constituants and detection of minor solutes. In: *Aphid-Plant Interactions: from Populations to Molecules.* Peters, D.C., Webster, J.A., and Chlouber, C.S., eds., Oklahoma State University, Stillwater, pp. 307.

Rahbé, Y., Delobel, B., Febvay, G., and Chantegrel, B. 1994. Aphid specific triglycerides in symbiotic and aposymbiotic *Acyrthosiphon pisum. Insect Biochemistry and Molecular Biology* **24**: 95–101.

Rahbé, Y., Delobel, B., Febvay, G., Nardon, C., and Nardon, P. 1993. Are aphid bacterial endosymbionts involved in the biosynthesis of aphid peculiar triglycerides? In: *Endocytobiology V.* Sato, S., Ishida, M., and Ishikawa, H., eds., Tübingen University Press, Tübingen, pp. 139–144.

Raven, J.A. 1983. Phytophages of xylem and phloem: a comparison of animal and plant sap-feeders. *Advances in Ecological Research* **13**: 136–234.

Sasaki, T., Aoki, T., Hayashi, H., and Ishikawa, H. 1990. Amino acid composition of the honeydew of symbiotic and aposymbiotic pea aphids *Acyrthosiphon pisum. Journal of Insect Physiology* **36**: 35–40.

Sasaki, T., Hayashi, H., and Ishikawa, H. 1991. Growth and reproduction of the symbiotic and aposymbiotic pea aphids, *Acyrthosiphon pisum* maintained on artificial diets. *Journal of Insect Physiology* **37**: 749–756.

Sasaki, T. and Ishikawa, H. 1995. Production of essential amino acids from glutamate by mycetocyte symbionts of the pea aphid, *Acyrthosiphon pisum. Journal of Insect Physiology* **41**: 41–46.

Whitehead, L.F. and Douglas, A.E. 1993. A metabolic study of *Buchnera*, the intracellular bacterial symbionts of the pea aphid *Acyrthosiphon pisum. Journal of General Microbiology* **139**: 821–826.

Wilkinson, T.L. and Douglas, A.E. 1995a. Aphid feeding, as influenced by disruption of the symbiotic bacteria: an analysis of the pea aphid (*Acyrthosiphon pisum*). *Journal of Insect Physiology* **41**: 635–640.

Wilkinson, T.L. and Douglas, A.E. 1995b. Why pea aphid (*Acyrthosiphon pisum*) lacking symbiotic bacteria have elevated levels of the amino acid glutamine. *Journal of Insect Physiology* **41**: 921–927.

Wright, J.P., Fisher, D.B., and Mittler, T.E. 1985. Measurement of aphid feeding rates on artificial diets using ^{3}H-inulin. *Entomologia Experimentalis et Applicata* **37**: 9–11.

Ziegler, H. 1975. Nature of transported substances. In: *Encyclopedia of Plant Physiology.* Pirson, A. and Zimmermann, M.H., eds., Springer Verlag, Berlin, pp. 59–100.