Lysine biosynthesis in *Chlorella* and *Euglena*: phylogenetic significance

Recent tracer studies with [4-^{14}C]aspartate have indicated a single over-all mode of lysine synthesis (presumably via α,ε-diaminopimelic acid) in bacteria, including strains of *Hydrogenomonas facilis*, *Pseudomonas fluorescens*, *Azotobacter agilis*, *Agrobacterium radiobacter*, *Alcaligenes faecalis*, *Escherichia coli*, *Micrococcus lysodeikticus*, *Arthrobacter globiformis*, *Bacillus subtilis*, and *Streptomyces griseus*[1]. However, certain fungi, in harmony with earlier results (*e.g.*, ref. [2]), showed a different pattern of labeling, indicative of a different path of lysine formation[1]. The consistency of these results and of certain others[3,4] encourages the view that biosynthetic pathways, as "biochemical organelles" representing appreciable periods of evolution, are characters of particular phylogenetic significance. The present findings suggest that such characters can reveal evolutionary relationships that are not otherwise readily discernible.

In Table I, the labeling patterns of *Chlorella vulgaris* and *Euglena gracilis* can be compared with those of *B. subtilis* and *Candida utilis*. It will be seen that in the case of *Chlorella* (as of *Bacillus*), with either DL-[4-^{14}C]aspartate or uniformly labeled L-[^{14}C]aspartate as tracer, protein lysine shows approximately the same specific activity as does protein aspartic acid. These results and similar ones obtained with *Chlorella pyrenoidosa* (ATCC No. 11469), as well as experiments with DL-[1-^{14}C]alanine as tracer, provide evidence that in *Chlorella* lysine is synthesized by the same general mechanism as it is in the bacteria[3,9,10], probably via α,ε-diaminopimelic acid as an intermediate. Such a role of α,ε-diaminopimelic acid is in accord with the demonstration of (relatively small amounts of) this amino acid in a strain of *Chlorella ellipsoidea*[11,12].

Additionally, Table I shows that *Euglena* differs in its mode of lysine synthesis from *Chlorella* and *Bacillus*, but resembles *Candida*. That the lysine pathway in

TABLE I

INCORPORATION OF TRACERS INTO PROTEIN AMINO ACIDS
(AS SPECIFIC ACTIVITY RELATIVE TO THE RESPECTIVE PROTEIN ASPARTIC ACID VALUES)

Organism	*Tracer*	*Aspartic acid*	*Lysine*
Chlorella vulgaris	DL-[4-^{14}C]Aspartate	100	98
Chlorella vulgaris	L-[^{14}C]Aspartate	100	96
Bacillus subtilis	DL-[4-^{14}C]Aspartate	100	104
Bacillus subtilis	L-[^{14}C]Aspartate	100	105
Euglena gracilis	DL-[4-^{14}C]Aspartate	100	< 10
Euglena gracilis	L-[^{14}C]Aspartate	100	52
Candida utilis	DL-[4-^{14}C]Aspartate	100	< 10
Candida utilis	L-[^{14}C]Aspartate	100	31

C. vulgaris, strain 211/11a, was kindly furnished by Dr. R. W. Krauss; *E. gracilis*, strain T (No. 752), was obtained from the Indiana University Culture Collection of Algae; and the *B. subtilis* (No. 6051) and *C. utilis* (No. 9950) strains were obtained from the American Type Culture Collection. The organisms were grown in 20 ml medium in 250-ml flasks either at 25° with illumination and occasional agitation (*Chlorella*, *Euglena*) or at 28° with continuous shaking (*Bacillus*, *Candida*). The chemically defined media used were slight modifications of known ones for *Chlorella*[5] and *Euglena*[6,7]; a glucose-salts medium was employed for *Bacillus* and *Candida*. Approx. 5–10 mg (20–40 μC) of the labeled aspartic acids were supplied per flask. For the determination of relative molar amounts of the three protein amino acids, experiments with D-[^{14}C]glucose as sole major carbon source were performed[2,8]. The cultures obtained were subjected to the harvesting, extraction, hydrolysis, paper chromatography, and counting procedures previously described, and specific activities were computed as before[2,8].

Euglena is similar to the one in fungi (such as *Candida* and *Neurospora*[13,14]) is also indicated by further isotope evidence: with L-[^{14}C]aspartate as tracer and with either DL-α-aminoadipic acid or DL-hexahomoserine as unlabeled supplement, the radioactivity in the protein lysine (but not in the protein aspartic acid or threonine) of *Euglena* is almost completely suppressed. An efficient utilization of these unlabeled supplements in the synthesis of lysine in this organism has thus been demonstrated. The substantial labeling of protein lysine with L-[^{14}C]aspartate, but not with DL-[4-^{14}C]aspartate, in *Euglena* and *Candida* is consistent with the view that these organisms have a citric acid cycle and utilize the succinyl moiety of α-ketoglutarate as a source of 4 of the 6 carbon atoms of lysine (*cf.* refs. [2] and [15]). Experiments with [2-^{14}C]acetate suggest that in *Euglena* the remaining two carbon atoms of lysine are derivable from acetate. In *Euglena* and *Candida* as well as in *Chlorella* and *Bacillus*, with either of the two labeled aspartates as tracer, protein threonine showed approximately the same specific activity as did protein aspartic acid.

From a phylogenetic point of view, it is noteworthy that, with respect to the character examined, *i.e.*, lysine synthesis, *Chlorella* appears to be related to the bacteria (and probably to the blue-green algae; *cf.* refs. [3, 4, 16]), but not to the fungi mentioned; in contrast, *Euglena* shows an affinity to the fungi rather than to the bacteria.

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