## Lysine synthesis and phylogeny: biochemical evidence for a bacterial-type endosymbiote in the protozoon *Herpetomonas (Strigomonas) oncopelti*

Recent tracer and enzyme studies have shown that lysine biosynthesis is a valuable character in the detection of certain evolutionary relationships[1-8]. All the bacteria[1,2], blue-green algae[2], green algae[3], and vascular plants[4,5] examined, as well as phycomycetes with anteriorly flagellated spores[6-8], appear to synthesize lysine in one general manner (via $\alpha,\varepsilon$-diaminopimelic acid), and phycomycetes with posteriorly flagellated or non-flagellated spores[6-8], ascomycetes[1,2], basidiomycetes[1,2], and euglenids[3] in another (via $\alpha$-aminoadipic acid). Reviews of [9,10] and references to [7] steps of lysine synthesis in Escherichia and Neurospora have appeared. The present results are concerned with the protozoon *Herpetomonas (Strigomonas) oncopelti*, a trypanosomid flagellate that has been reported[11,12] to grow on an exceptionally simple, lysine-free medium. Evidence has now been obtained that the relative nutritional self-sufficiency attributed to this protozoon may be only apparent.

TABLE I

INCORPORATION OF TRACERS INTO PROTEIN AMINO ACIDS OF *H. oncopelti*

The experiments were carried out in the same general manner as before[4]. The strain of *H. oncopelti* was kindly furnished by Dr. F. G. WALLACE. The protozoon was cultivated during 6 days at 25°, without agitation, in chemically defined medium[11] supplemented with sodium L-glutamate[13] (2.5 mg/ml) and the desired tracer. For the determination of relative molar amounts of protein threonine and lysine, uniformly labeled major carbon sources were used. The results are expressed as specific radioactivity (on a molar basis) relative to the respective protein threonine values.

| *Tracer* | *Threonine* | *Lysine* |
|---|---|---|
| DL-[3-$^{14}$C]Aspartate | 100 | 112 |
| DL-[4-$^{14}$C]Aspartate | 100 | 104 |
| DL-[1-$^{14}$C]Alanine | 100 | 140 |

As illustrated in Table I, tracer experiments with *H. oncopelti* gave a labeling pattern corresponding to the bacterial kind[1,4] of lysine pathway, *i.e.*, via $\alpha,\varepsilon$-diaminopimelic acid. (Earlier tracer studies with this protozoon[12] did not make it possible to determine which kind of lysine path is used; *cf.* data on Neurospora[14] and Bacillus[3].) Since the euglenids[3] and higher fungi[1,2] (to which *H. oncopelti* may have phylogenetic affinities[15]) employ the $\alpha$-aminoadipic acid - lysine path, and since *H. oncopelti* is nutritionally atypical[11,15-17], the possibility suggested itself that the labeling pattern of the latter organism (Table I) is due to lysine synthesis by a bacterial-type endosymbiote. Consonant with this possibility is the finding[18] that *H. oncopelti* contains so-called bipolar bodies which are osmotically fragile structures of bacterial dimensions (*cf.* general discussions of presumable protozoon-bacterium complexes[19,20]).

To test the endosymbiote hypothesis, experiments were performed on the localization of diaminopimelic decarboxylase, an enzyme of lysine synthesis first shown[21] in *E. coli*. As is seen in Table II, decarboxylase activity is demonstrable in sonic extracts of whole *H. oncopelti* cells and also in a preparation containing bipolar bodies and debris, but free of soluble protozoan cytoplasm. Experiments with preparations from which the debris was largely removed by fractional centrifugation indicated that the decarboxylase activity is indeed associated with the bipolar bodies. It is

TABLE II

DIAMINOPIMELIC DECARBOXYLASE IN *H. oncopelti* AND BIPOLAR-BODY PREPARATIONS

Protozoa grown on peptone–liver extract–glucose[16] were used. The particle fraction (bipolar bodies and fine debris) was prepared from the (0.25 *M* sucrose-containing) supernatant liquid obtained after treatment of the protozoa in a Mickle Disintegrator[18] and centrifugation at 900 × *g*. Enzyme incubations with *meso*-α, ε-diaminopimelic acid were performed as before[5]. Lysine (in the presence of substrate) was determined spectrophotometrically[22] and with *E. coli* mutant ATCC 12408. Decarboxylase activity is given as μmoles L-lysine/mg protein/h.

| *Preparation* | *Activity* |
|---|---|
| *H. oncopelti*, whole cells | 0.0 |
| *H. oncopelti*, sonically disrupted | 0.2 |
| Particle fraction, as is | 0.2 |
| Particle fraction, sonically disrupted | 0.7 |

suggested that the bipolar bodies are (presumably cell-wall-deficient) bacteria or bacteria-like organisms that furnish lysine and probably other metabolites to the protozoon, which in turn provides osmotic protection and perhaps other advantages for the endosymbiote.

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