Carboxypeptidase Activity in Human Mycoplasmas

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*Mycoplasma salivarium* produced citrulline, ammonia, and ATP from *N*-benzoylglycyl-l-arginine. The activity was inhibited by EDTA and was therefore concluded to be due to an arginine-specific carboxypeptidase. The activity was also found to exist in *M. orale*, *M. buccale*, *M. fauclae*, and *M. hominis*.

Proteolytic activity has been demonstrated in *Mycoplasma salivarium* (10). The hydrolytic activity toward casein is weak and is inhibited by o-phenanthroline and EDTA. Therefore, the activity is presumed to be due to metallo-exopeptidases such as aminopeptidases or carboxypeptidases. Recently, aminopeptidase activity was found to exist in *M. salivarium* and was separated from caseinolytic activity by gel filtration (11).

The present study, designed to examine human mycoplasmas for carboxypeptidase activity, suggested that an arginine-specific carboxypeptidase existed in nonfermentative human mycoplasmas.

Most of the *Mycoplasma* strains tested were *Mycoplasma salivarium* ATCC 23064. Other mycoplasmas used for comparative purposes are listed in Table 1.

The media used were PPP0 broth (Difco Laboratories, Detroit, Mich.) with 1% (vol/vol) PPP0 serum fraction (SF medium) and the liquid medium of Hayflcyk (5) supplemented with 1% (wt/vol) glucose (G medium) or 1% (wt/vol) arginine hydrochloride (A medium).

*M. fermentans* and *M. pneumoniae* were grown in G medium, *M. salivarium* was grown in both A and SF media, and the other mycoplasmas were grown in A medium. Cells were harvested by centrifuging the cultures at 15,000 × g for 15 min, washed three times, and suspended in a small volume of 0.25 M NaCl (cell suspensions [CS]). CS were kept at −70°C until used. When used, CS were thawed at 37°C and diluted appropriately with distilled water. Protein concentrations of CS were determined by the method of Lowry et al. (6).

Ninhydrin-positive substances (NPS) produced from *N*-benzoylglycyl-l-arginine (Bz-Gly-Arg), benzoylglycyl-l-lysine (Bz-Gly-Lys), and benzoylglycyl-l-histidine-l-leucine (Bz-Gly-His-Leu) (Protein Research Foundation, Osaka, Japan) were assayed by the colorimetric ninhydrin method described by Tsunasawa et al. (8) and expressed as the absorbance difference at 570 nm between the reaction mixtures with and without substrate (ΔA570), measured by using a 1-cm cuvette. The buffer used was 0.1 M potassium phosphate buffer (pH 6.5).

Table 1. NPS production from Bz-Gly-Arg by CS of human mycoplasmas

<table>
<thead>
<tr>
<th>Mycoplasma tested</th>
<th>Activity a</th>
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</thead>
<tbody>
<tr>
<td><em>M. pneumoniae</em> IID817</td>
<td>0.02</td>
</tr>
<tr>
<td><em>M. fermentans</em> IID812</td>
<td>0.03</td>
</tr>
<tr>
<td><em>M. fauclae</em> IID996</td>
<td>4.12</td>
</tr>
<tr>
<td><em>M. buccale</em> IID802</td>
<td>5.12</td>
</tr>
<tr>
<td><em>M. salivarium</em> ATCC 23064</td>
<td>2.36</td>
</tr>
<tr>
<td><em>M. orale</em> ATCC 15539</td>
<td>2.95</td>
</tr>
<tr>
<td><em>M. hominis</em> IID801</td>
<td>0.72</td>
</tr>
</tbody>
</table>

* Corresponding author.

a. Obtained from the Institute of Medical Science, University of Tokyo, Tokyo, Japan.

b. Expressed as the mean of three determinations of the ΔA570/5 min per mg of protein, measured by using a 1-cm cuvette.

c. M. salivarium and the other nonfermentative mycoplasmas were grown in a medium.
On the basis of these results, it was presumed that no sooner than arginine had been produced from Bz-Gly-Arg by an arginine-releasing enzyme such as a carboxypeptidase, arginine was hydrolyzed by the enzymes in the arginine dihydrolase pathway. This was considered to be the reason that arginine was not detected in the reaction mixtures. To validate this speculation, we examined the effect of EDTA on NPS production, because this chelator is known to inhibit carboxypeptidases (4). NPS production was inhibited by EDTA (Fig. 3), suggesting that carboxypeptidase activity existed in *M. salivarium*.

The other human mycoplasmas were also examined for NPS production from Bz-Gly-Arg. Carboxypeptidase activity was found to exist in nonfermenters (*M. orale, M. buccale, M. faucale, and M. hominis*) but not in fermenters (*M. fermentans and M. pneumoniae*) (Table 1). Incidentally, mycoplasmas are divided into fermentative or nonfermentative species on the basis of acid production from carbohydrate. *M. fermentans* (2) is a fermentative species but is also a nonfermentative species (2, 7) and is known to possess the arginine dihydrolase pathway by which arginine is catabolized to ATP. Therefore, cells of the organism grown in A medium were also examined for carboxypeptidase activity. This activity (ΔA570/5 min per mg of protein, 0.02) was demonstrated to be almost equivalent to that (0.03, Table 1) in cells grown in G medium.

This is the first report of the existence of an arginine-specific carboxypeptidase in nonfermentative human

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**FIG. 2.** ATP formation from ADP through the metabolism of arginine or Bz-Gly-Arg. CS diluted appropriately with distilled water were incubated with ADP and arginine or Bz-Gly-Arg at 37°C for 0, 20, 40, and 80 min. ATP contents were corrected with the contents obtained in control experiments. Symbols: ○, arginine; ▲, Bz-Gly-Arg.

**FIG. 3.** Effect of EDTA on NPS production from Bz-Gly-Arg by CS of *M. salivarium*. CS (34 μg of protein) were preincubated in the absence (○) or presence of 1 mM EDTA (▲) or 2 mM EDTA (△) at 37°C for 10 min, and then NPS production was assayed at regular intervals by the method described in the text.
mycoplasmas, although the activity is already known to exist in *Acholeplasma laidlawii*, a fermentative species (9). As indicated above, nonfermenters catabolize arginine and possess an arginine-specific aminopeptidase (1, 11). Judging from the arginine specificity of the carboxypeptidase and the aminopeptidase and from the well-known significance of the arginine dihydrolase pathway in nonfermentative mycoplasmas, these peptidases seem to be naturally occurring enzymes, perhaps functioning to supply arginine to the pathway. Further studies are in progress in our laboratory to purify and characterize the enzymes.

LITERATURE CITED