Histidine decarboxylases from bacteria that colonise the human respiratory tract

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Summary. We investigated whether production of histamine by bacteria isolated from sputum of patients with infective lung diseases could be attributed to the presence of histidine decarboxylase (HD). Twenty gram-positive and 20 gram-negative organisms were studied for their ability to decarboxylate ^{14}C -histidine *in vitro* over the pH range 4.5–7.5. Of the bacteria investigated, lysates from the gram-negative species *Haemophilus influenzae*, *H. parainfluenzae*, *Moraxella (Branhamella) catarrhalis* and *Pseudomonas aeruginosa* liberated $^{14}\text{CO}_2$ and histamine from ^{14}C -histidine in the presence of the cofactor pyridoxal phosphate. In contrast, results obtained in the absence of cofactor were similar to those of negative (lysate-free) controls suggesting that the HD enzymes of these species resembled those previously described in other gram-negative bacteria. No HD activity was detected over this pH range in lysates from gram-positive species. This finding correlated with earlier observations that these gram-positive organisms did not produce histamine *in vitro*.

Introduction

Histamine production by gram-positive and gram-negative bacteria has been reported by several authors and has been associated with food spoilage by species such as *Salmonella typhimurium*, *Lactobacillus buchnerii* and *Morganella morganii*.¹ Although many of the bacteria that produce histamine by decarboxylation of histidine inhabit the gastrointestinal tracts of animals and man,² recent studies have demonstrated that several gram-negative species which may be found in the respiratory tract are also capable of histamine production.^{3,4} These include *Haemophilus parainfluenzae*, *H. influenzae* and *Pseudomonas aeruginosa*. In contrast, none of the gram-positive bacteria isolated from similar sites investigated so far (*Staphylococcus aureus*, *Streptococcus pneumoniae*, α -haemolytic streptococci and coagulase-negative staphylococci) have been found to produce histamine.⁴

Histamine production by bacteria is usually due to the presence of a functional histidine decarboxylase (HD) that converts histidine to histamine and CO_2 . Some other decarboxylase enzymes, notably arginine decarboxylase, can also decarboxylate small amounts of histidine.^{5,6} HDs are widespread, occurring in both mammalian⁷ and bacterial cells.⁸ They have been divided into two major groups—those that require pyridoxal phosphate (pyridoxal-P) as a co-enzyme and those that contain a covalently-bound pyruvoyl residue

at the active site and do not require the cofactor.⁹ The former class of enzymes has been found only in gram-negative bacteria whereas the latter occur in gram-positive organisms.^{7,8} However, both classes of enzyme are highly specific for histidine as substrate and metabolise this compound at similar rates.⁸ Several authors have demonstrated that optimal activity of bacterial HDs depends on the pH of the surrounding medium, with enzymes from gram-positive and gram-negative bacteria functioning optimally at pH 4.5 and 6.5 respectively.^{5,7,8,9} In view of this pH-dependency of HDs the aim of this study was to investigate whether the previously observed histamine production by certain bacterial species that may inhabit the respiratory tract was associated with a functional HD and whether activity of any enzymes present was affected by changes in pH.

Materials and methods

Isolation and disruption of bacteria

Isolates studied (table) were found in sputum obtained from patients admitted to hospital because of acute exacerbations of chronic bronchitis, pneumonia or cystic fibrosis. Species were identified by standard microbiological techniques as described previously.⁴ Organisms were suspended in 2 ml of 0.2 M sodium phosphate buffer, pH 7.0, and homogenised by sonication on ice (Sonifier® cell disrupter, Branson Sonic Power Company) for 3 min. Sonicates were examined by light microscopy to ensure that no

Table. Bacteria studied which were isolated from sputum of patients with infective lung disease

Strains studied (n)	Diagnosis of patient
Gram-negative (20)	
<i>Haemophilus influenzae</i> (5)	Chronic bronchitis, pneumonia
<i>Moraxella</i> (<i>Branhamella</i>) <i>catarrhalis</i> (5)	Chronic bronchitis
<i>Haemophilus parainfluenzae</i> (5)	Chronic bronchitis
<i>Pseudomonas aeruginosa</i> (5)	Cystic fibrosis
Gram-positive (20)	
<i>Staphylococcus aureus</i> (5)	Chronic bronchitis, pneumonia
<i>Streptococcus pneumoniae</i> (5)	Pneumonia
α -Haemolytic streptococci (5)	Chronic bronchitis
Coagulase-negative staphylococci (5)	Chronic bronchitis

intact bacteria remained. Staphylococci were not disrupted by this procedure but were lysed completely by adding lysostaphin (Sigma) 5 $\mu\text{g/ml}$ and incubating for 15 min at 37°C.

Detection of HD activity

HD activity was measured by the method of Beaven *et al.*¹⁰ Bacterial lysates (250 μl) which were standardised to be equivalent to material from *c.* 1×10^6 bacteria (based on viable counts performed on suspensions before homogenisation) were mixed with a combination of radiolabelled (50 nCi/ml) and unlabelled (2.5×10^{-4} M)L-histidine. All reaction mixtures were adjusted to a final volume of 1.25 ml with buffer of appropriate pH and the pH was checked before incubation. Experiments with lysates from both gram-negative and gram-positive bacteria were performed in the presence of 10^{-5} M pyridoxal phosphate. Additionally, experiments with the gram-negative species were performed in the absence of pyridoxal phosphate. For investigations of the effect of pH over the range 4.0–6.0, sodium citrate phosphate buffer of final molarity 0.2 M was used whereas sodium phosphate buffer (0.2 M) was used in experiments on the effect of pH over the range 6.0–8.0. Negative controls were prepared with the same incubation mixture but with buffer of an appropriate pH substituted for the bacterial lysate. Positive controls were prepared with commercial preparations of pyruvoyl- and pyridoxal phosphate-dependent HDs from *Clostridium perfringens*¹¹ and *Escherichia coli*,¹² (Sigma) respectively, each used at 0.01 unit/ml.

All incubation was in airtight, polypropylene tubes, overnight, in a moist box at 37°C and any CO_2 generated was absorbed into 200 μl of hyamine hydroxide contained in a plastic vial placed in each tube. After incubation, the vials were transferred into 5 ml of scintillant (Beckman Company, High Wycombe) and the radioactive CO_2 ($^{14}\text{CO}_2$) fraction was assayed in a Beckman scintillation counter.

$^{14}\text{CO}_2$ production by bacterial extracts was calculated according to the following formula:

$$\text{Amount of histidine added (50 nCi)} = \frac{200\,000 \text{ cpm}}{0.0045 \text{ } \mu\text{mol/ml}}.$$

Therefore, $^{14}\text{CO}_2$ produced ($\mu\text{mol/ml}$) =

$$\frac{\text{cpm}}{200\,000} \times 0.0045.$$

Histamine in the incubation mixtures was detected by a reversed phase high performance liquid chromatographic technique developed in our laboratory and described previously.¹³

Statistical analysis

Histamine and $^{14}\text{CO}_2$ generation were compared by unpaired Student's *t* and Mann-Whitney U tests on the logarithmically ($\log_{10}$) transformed data. Correlation between histamine and $^{14}\text{CO}_2$ generation was assessed by linear regression analysis.

Results

Correlation between $^{14}\text{CO}_2$ release and histamine production

A highly significant correlation ($p < 0.001$, $r = 0.95$) was observed between histamine production and CO_2 release by lysates from gram-negative bacteria (fig. 1).

$^{14}\text{CO}_2$ production by commercial preparations of HD

$^{14}\text{CO}_2$ production by the commercially available HDs from *C. perfringens* and *E. coli* is shown in fig. 2. HD from *E. coli* produced significantly ($p < 0.001$) greater amounts of $^{14}\text{CO}_2$ in the presence of pyridoxal phosphate than in the mixture without pyridoxal phosphate or the control mixture. In contrast, HD from *C. perfringens* generated $^{14}\text{CO}_2$ in the absence of pyridoxal phosphate in amounts similar to those produced by the *E. coli*-derived enzyme in the presence of the cofactor.

Production of $^{14}\text{CO}_2$ by clinical isolates

The ability of gram-negative bacteria to liberate $^{14}\text{CO}_2$ from ^{14}C -histidine is shown in fig. 3. Production of CO_2 was significantly greater in lysate-containing tubes supplemented with pyridoxal phosphate than in control tubes ($p < 0.001$). However, generation of CO_2 in the absence of pyridoxal phosphate was not significantly different from amounts produced by control mixtures. In contrast, lysates from the gram-positive species, *S. aureus*, *Str. pneumoniae*, α -haemolytic streptococci and coagulase-negative staphylococci did not metabolise histidine. Lysates from these organisms, which were supple-

Fig. 1. Correlation between $^{14}\text{CO}_2$ release and histamine production by lysates from 20 gram-negative bacteria.

Fig. 3. $^{14}\text{CO}_2$ production by lysates from gram-negative bacteria in the presence ($\blacktriangle$) and absence ($\triangle$) of pyridoxal phosphate, compared with release of $^{14}\text{CO}_2$ from control mixture ($\bullet$). Results are expressed as log means and SEM ($n=20$) (*, $p < 0.001$).

Fig. 2. $^{14}\text{CO}_2$ production by commercial preparations of HD from *E. coli* in the presence ($\bullet$) or absence ($\circ$) of pyridoxal phosphate, and from *C. perfringens* ($\blacksquare$), compared with release of $^{14}\text{CO}_2$ from HD-free control mixture ($\times$). Results are expressed as log means and SEM ($n=6$) (*, $p < 0.01$).

mented with pyridoxal phosphate, did not produce significantly larger quantities of $^{14}\text{CO}_2$ than control mixtures of any pH value studied (fig. 4).

Discussion

It has been determined previously that certain gram-negative, but not gram-positive, bacteria that may

Fig. 4. $^{14}\text{CO}_2$ production by lysates from gram-positive bacteria after sonication ($\blacksquare$), lysostaphin treatment of staphylococcal isolates ($\blacklozenge$) and release of $^{14}\text{CO}_2$ by negative control ($\blacktriangle$) ($n=20$).

colonise the respiratory tracts of patients suffering from chronic bronchitis, cystic fibrosis and pneumonia, are capable of generating histamine *in vitro*.⁴ The results of the present study demonstrated that gram-negative but not gram-positive species isolated from sputum of similar patients possess HD activity and, therefore, have the capacity to metabolise histidine to CO_2 and histamine. Furthermore, the observation that HD activity in the gram-negative

organisms was pyridoxal phosphate-dependent over the pH range examined indicated that their HDs were similar to those previously described among gram-negative species by other workers.^{5,9}

Some bacterial amino-acid decarboxylases, including the arginine decarboxylases of species such as *S. typhimurium* and *E. coli*,⁶ have been shown to be capable of metabolising histidine in addition to their natural substrate. In contrast, HDs have been shown to be highly specific for histidine as their substrate. Although we have used crude bacterial lysates that may contain several decarboxylase enzymes that might metabolise histidine to some extent, the results obtained with lysates not supplemented with pyridoxal phosphate indicate that the metabolism of histidine observed in these studies is likely to be due to HDs. The demonstration that a small amount of CO₂ was

generated in lysate-free "negative" controls indicated that some spontaneous degradation of histidine must also have occurred under these assay conditions.

In marked contrast to HDs of gram-negative bacteria, several studies have demonstrated that the activity of enzymes from gram-positive bacteria, including *L. buchnerii*, *Lactobacillus* 30a and *C. perfringens*, depends on the presence of a pyruvoyl moiety.^{2,9,11} Our observation that the gram-positive bacteria studied were unable to metabolise histidine, even in the presence of pyridoxal phosphate, could be explained either by the occurrence of inactive forms of HD in these organisms or by complete absence of the enzyme.

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