EFFECTS OF HYDROXYLAMINE, SODIUM NITRITE, AMMONIUM SULFATE AND ETHANOL ON DIAMINE OXIDASE OF HOG KIDNEY

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Abstract—Enzymic properties of diamine oxidase (DAO) and histaminase were compared. The enzymic activities of a partially purified preparation from hog kidney were determined by measuring oxygen consumption using cadaverine and histamine as substrates for DAO and histaminase, respectively. Differences were found in the effects of various reagents on the two activities. Although NH₂OH inhibited to a similar extent the activities of DAO and histaminase, NaNO₂, (NH₄)₂SO₄ and C₂H₅OH affected the two activities differently. Moreover, NH₂OH and NaNO₂ had no effects on the pS curves of the two enzymes. (NH₄)₂SO₄ and C₂H₅OH had no effect on the pS maximum of histaminase, but shifted the pS maximum to a higher concentration of cadaverine. Furthermore, marked differences in the mechanisms of action of DAO and histaminase were apparent from the effects of NH₂OH, NaNO₂, (NH₄)₂SO₄ and C₂H₅OH on the two activities as measured from Lineweaver-Burk plots. These results indicate that the enzymic properties of DAO and histaminase are different and suggest that they are probably different enzymes.

The problem of whether the activities of histaminase and diamine oxidase (DAO) are due to different enzymes has long been debated (1–3). Zeller (4) found that an enzyme preparation from hog kidney oxidized not only histamine but also cadaverine and putrescine, and claimed that activity was due to a single enzyme. Mondovi et al. (5–7) found that the ratio of the activities of their preparation for cadaverine and histamine remained constant during partial purification and concluded that these activities were due to a single enzyme. Davison (8) reached a similar conclusion from studies on heat inactivation and the effects of inhibitors on the two activities. However, Kapeller-Adler and MacFarlane (9), and Uozumi et al. (10) concluded that DAO and histaminase activities are due to different enzymes, because the extents of purification of the activities for cadaverine and histamine differed at different purification steps, and because a purified preparation with activity for histamine did not oxidize cadaverine. Thus no definite conclusion has yet been reached on whether the activities are due to one or two enzymes. Recently Suetsugu (11), using partially purified enzyme from hog kidney, studied the multiplicity of the enzyme and concluded that there are two enzymes which have very similar enzymic properties but which are affected in slightly different ways by certain inhibitors.

In this work partially purified enzyme from hog kidney, was used with cadaverine and histamine as substrates, and the effects of hydroxylamine (NH₂OH), sodium nitrite (NaNO₂), ammonium sulfate [(NH₄)₂SO₄] and ethanol (C₂H₅OH) on the activities of DAO and histaminase were investigated.
MATERIALS AND METHODS

Enzyme purification

The enzyme was partially purified from hog kidney cortex by the method of Suetsugu (11).

Measurement of enzymic activity

Enzymic activity was measured using a Clark oxygen electrode following the method of Sho et al. (12). A mixture of 1.0 ml of 0.1 M phosphate buffer (pH 7.1), enzyme and distilled water to a volume 2.9 ml was equilibrated at 38°C and then 0.1 ml of substrate solution was added to the mixture. Oxygen consumption was measured for two to three min after addition of the substrate, and corrections were made for non-enzymic oxygen consumption. Unless otherwise stated, 1 x 10^{-3} M cadaverine and 1 x 10^{-4} M histamine were used as substrates.

RESULTS

Effects of NH_{2}OH, NaNO_{2}, (NH_{4})_{2}SO_{4} and C_{2}H_{5}OH on the two activities

The effects of NH_{2}OH were investigated with cadaverine and histamine as substrates. Addition of NH_{2}OH 1 x 10^{-5} M inhibited both activities completely. At a concentration of 1 x 10^{-6} M, NH_{2}OH inhibited both activities by 50–75% while at a concentration of 1 x 10^{-7} M, it had no effect on either activity. Thus there was no significant difference in the inhibitions of the two activities by NH_{2}OH.

![Fig. 1. Effects of NaNO_{2} on partially purified enzyme from hog kidney. Cadaverine 1 x 10^{-3} M (open circles) and histamine 1 x 10^{-4} M (solid circles) were used as substrates. Abscissa: negative logarithm of molar concentration of NaNO_{2}. Ordinate: percentage of control DAO activity.](image1)

![Fig. 2. Effects of (NH_{4})_{2}SO_{4} on partially purified enzyme from hog kidney. Cadaverine 1 x 10^{-3} M (open circles) and histamine 1 x 10^{-4} M (solid circles) were used as substrates. Abscissa: negative logarithm of molar concentration of (NH_{4})_{2}SO_{4}. Ordinate: as in Fig. 1.](image2)
As shown in Fig. 1, with cadaverine as substrate, at concentrations below $1 \times 10^{-2}$ M, NaNO$_2$ scarcely caused any inhibition, while at $1 \times 10^{-1}$ M it caused slight inhibition. With histamine as substrate, $1 \times 10^{-2}$ M NaNO$_2$ caused activation but $1 \times 10^{-1}$ M caused slight inhibition of activity. At concentrations below $1 \times 10^{-3}$ M, the effects of NaNO$_2$ on the activities with cadaverine and with histamine were similar.

Effects of (NH$_4$)$_2$SO$_4$ were also investigated with cadaverine as substrate and the results are shown in Fig. 2. Strong inhibition of the activity was observed with $1 \times 10^{-1}$ M (NH$_4$)$_2$SO$_4$, while stimulation with $1 \times 10^{-2}$ M, slight stimulation with $1 \times 10^{-3}$ M to $1 \times 10^{-5}$ M and no effect with less than $1 \times 10^{-6}$ M (NH$_4$)$_2$SO$_4$ were observed. With histamine as substrate, inhibition was observed at $1 \times 10^{-1}$ M and $1 \times 10^{-2}$ M (NH$_4$)$_2$SO$_4$ and there was no effect at $1 \times 10^{-3}$ M (NH$_4$)$_2$SO$_4$. Thus at a concentration of $1 \times 10^{-2}$ M, (NH$_4$)$_2$SO$_4$ had very different effects of the activities with cadaverine and with histamine; it stimulated the former, and inhibited the latter.

Remarkable effects on both activities were observed with C$_2$H$_5$OH. As shown in Fig. 3, C$_2$H$_5$OH increased the activities with both cadaverine and histamine. It had the most prominent effect at a concentration of about $1 \times 10^{-1}$ M, increasing both activities to about 200% of the control values.

**Effects of NH$_2$OH, NaNO$_2$, (NH$_4$)$_2$SO$_4$ and C$_2$H$_5$OH on the pS curves**

Results of the effects of $3 \times 10^{-7}$ M NH$_2$OH on the pS curves with the two substrates
are shown in Fig. 4. With both histamine and cadaverine $3 \times 10^{-7}$ M NH$_2$OH inhibited activity at all concentrations of substrate used in this experiment and there was no change in the pS maximum.

In contrast, NaNO$_2$ increased activity at all concentrations of cadaverine and particularly at high concentrations, and the pS maximum did not change. Similarly, NaNO$_2$ increased activity slightly at all concentrations of histamine used and here again the pS maximum remained unchanged (Fig. 5).

Most significant differences between cadaverine and histamine as substrate were ob-
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Fig. 6. Effects of (NH₄)₂SO₄ (1 x 10⁻² M) on the pS-curves. Effect of (NH₄)₂SO₄ on the pS-curve with cadaverine (left) and histamine (right). Abscissas and ordinates: as in Fig. 4.

Fig. 7. Effects of C₂H₅OH (3 x 10⁻¹ M) on the pS-curves. Effect of C₂H₅OH on the pS-curve with cadaverine (left) and histamine (right). Abscissas and ordinates: as in Fig. 4.

tained using (NH₄)₂SO₄. The results are shown in Fig. 6. (NH₄)₂SO₄ caused a marked increase in activity with concentrations of more than 1 x 10⁻³ M cadaverine and slight increase in activity with lower concentrations (Fig. 6, left). Moreover, (NH₄)₂SO₄ shifted the pS maximum from 1 x 10⁻³ M to 1 x 10⁻² M. On the other hand, (NH₄)₂SO₄ inhibited activity at all concentrations with histamine tested and did not alter the pS maximum (Fig. 6, right).

As shown in Fig. 7, C₂H₅OH caused a marked increase in activity at all concentrations with cadaverine tested and the pS maximum shifted from 1 x 10⁻³ M to 1 x 10⁻².5 M by the addition of C₂H₅OH (Fig. 7, left). On the other hand, C₂H₅OH increased in activity at all concentrations with histamine tested but did not change the pS maximum (Fig. 7, right).
Mechanisms of action of NH$_2$OH, NaNO$_2$, (NH$_4$)$_2$SO$_4$ and C$_2$H$_5$OH on the activities

The mechanism of inhibitory action of NH$_2$OH was studied from Lineweaver-Burk plots and the Km values using the two substrates were estimated. As shown in Fig. 8, in the absence of NH$_2$OH, the Km values were estimated as $2 \times 10^{-4}$ M for cadaverine and $6.7 \times 10^{-5}$ M for histamine. With cadaverine and histamine as substrates, as can be seen in the Fig. 8, there is a marked difference in the mechanisms of inhibition of the two activities by $3 \times 10^{-7}$ M NH$_2$OH.

Results on the activations of the two activities by NaNO$_2$ are shown in Fig. 9. Addition of $1 \times 10^{-4}$ M NaNO$_2$ caused activation with both cadaverine and histamine. The Lineweaver-Burk plots of the activities with and without NaNO$_2$ gave straight lines which intersected on the horizontal axis with cadaverine as substrate and on the vertical axis with histamine as substrate.

![Fig. 8](image1.png)

**Fig. 8.** Effects of NH$_2$OH on Lineweaver-Burk double reciprocal plots of activity in the presence and absence of NH$_2$OH ($3 \times 10^{-7}$ M).

![Fig. 9](image2.png)

**Fig. 9.** Effects of NaNO$_2$ on Lineweaver-Burk double reciprocal plots of activity in the presence and absence of NaNO$_2$ ($1 \times 10^{-4}$ M).
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FIG. 10. Effects of (NH₄)₂SO₄ on Lineweaver-Burk double reciprocal plots of activity in the presence and absence of (NH₄)₂SO₄ (1 x 10⁻² M).

The results suggest that the activation is non-competitive with cadaverine, although such activation is competitive with histamine. Thus the mechanisms of the activation of the two activities by NaNO₂ were also different.

As shown in Fig. 10, the Lineweaver-Burk plots of activity with and without (NH₄)₂SO₄ gave two straight lines which did not intersect on either axis with cadaverine as substrate but did intersect on the vertical axis when histamine was used as substrate. Thus there appears to be a significant difference in the mechanisms of inhibition of the two activities by (NH₄)₂SO₄.

Results of the effect of 3 x 10⁻¹ M C₂H₅OH on both activities are shown in Fig. 11. The two straight lines obtained with and without C₂H₅OH intersected on the horizontal axis with cadaverine as substrate and on the vertical axis with histamine as substrate. Thus the actions of C₂H₅OH on the two activities were also different.

DISCUSSION

There have been many reports on the problem of whether DAO and histaminase activities are due to a single enzyme. Kapeller-Adler and MacFarlane (9) partially purified
the enzyme(s) from hog kidney and compared the activities using cadaverine and histamine as substrates at each step of purification. They concluded that the two activities might be due to different enzymes, since their final preparation did not oxidize cadaverine. In contrast, Mondovi et al. (7) purified the enzyme from hog kidney and claimed that the activities were due to a single enzyme because the ratio of the activities with cadaverine and histamine remained constant during purification. Buffoni (13) concluded that the difference between the results of Kapeller-Adler and MacFarlane and of Mondovi was due to the different method used to measure the activity. Buffoni considered that the results of Mondovi et al., obtained by measuring oxygen consumption were the more accurate. Recently Suetsugu (11) using a partially purified enzyme preparation observed a difference in the heat stabilities of the activities with cadaverine and with histamine. He also found a marked difference in the inhibitions of the two activities by AgNO₃. From these, he concluded that DAO and histaminase may be different enzyme.

In this work, the effects of NH₂OH, NaNO₂, (NH₄)₂SO₄ and C₂H₅OH on the activities were examined. In the present work, NH₂OH strongly inhibited the activities using both cadaverine and histamine. Thus the effects of NH₂OH on the two amine oxidases, DAO and MAO, are different and these effects on both enzymes may depend upon the difference in the co-factor or the enzyme molecule. Since, NH₂OH inhibits DAO but activates MAO (14). With cadaverine as substrate, NH₂OH was found to be non-competitive while with histamine it was competitive. This difference in the mechanisms of inhibition of the activities by NH₂OH indicates that the activities are due to two enzymes. Furthermore, NaNO₂ caused a marked increase in activity with cadaverine but little increase in activity with histamine and its effect was non-competitive with cadaverine and competitive with histamine. This difference in the effects of NaNO₂ on the two activities also indicates the possibility that the activities are the result of two enzymes. Moreover, (NH₄)₂SO₄ was found to activate activity with cadaverine as substrate but inhibit that with histamine. Thus (NH₄)₂SO₄ revealed opposite effects with different substrates. C₂H₅OH increased activity with both substrates, however, it was competitive with histamine but not with cadaverine. Marked differences were thus observed in the effects of NaNO₂, (NH₄)₂SO₄ and C₂H₅OH on the two activities. Moreover, the pS maxima of DAO shifted on addition of (NH₄)₂SO₄ and C₂H₅OH, with cadaverine as substrate but not with histamine as substrate. Judging from kinetic experiments, the effects of these reagents also indicated a significant difference in the mechanisms of action with both substrates. Although it would appear that the two activities are due to two enzymes, a complete separation of the two enzymes is required before final conclusions can be drawn.

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