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*Short Communication*

# Evidence for Enzymatic ADP-Ribosylation to Histidine and Related Dipeptides

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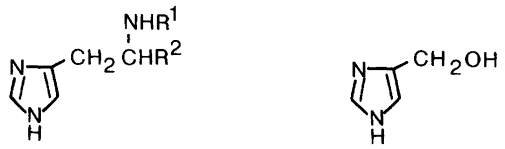
Interestingly mammalian tissue (spleen, brain, etc.)-derived $NAD^+$ glycohydrolase (NADase) [EC 3.2.2.5] catalyses, besides the ordinary hydrolysis of a quaternary nicotinamide–ribose glycosidic linkage of $NAD^+$, transfer of ADP-ribosyl groups from $NAD^+$ to appropriate azoles as well as to pyridine derivatives. This enzyme has been found to exhibit an ADP–ribosyltransferase-like activity toward various types of target substrate, e.g., pyridines,[1,2] indazoles,[3] 1,2,4-triazoles,[4] etc. On the other hand, ADP-ribosyltransferase activity specific for a proteinic substrate has been detected in human erythrocytes.[5] Thus, in connection with haemocyte-derived enzymatic activity, it is of significance to examine further the transferase-like action of tissue-derived NADase on the constituents of protein, azole-type amino acid and related peptides.

In this study, we investigated porcine-brain NADase-catalysed ADP-ribosylation for histidine and related compounds together with some histidine-containing dipeptides and obtained $^1H$ NMR spectral evidence for their undergoing ADP-ribosylation.



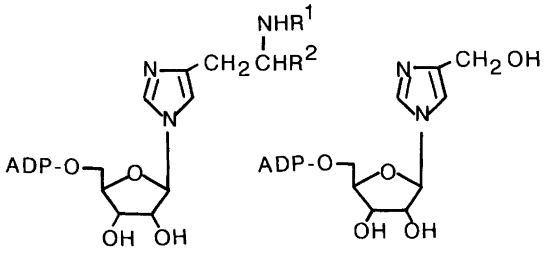
**3**

**1** $R^1$=H ; $R^2$=$CO_2H$
**2** $R^1$=H ; $R^2$=$CH_2OH$
**4** $R^1$=H ; $R^2$=$CONHCH_2CO_2H$
**5** $R^1$=$COCH_2NH_2$ ; $R^2$=$CO_2H$
**6** $R^1$=H ; $R^2$=$CONHCH(CH_3)CO_2H$

## Results and discussion

The enzymatic reaction was examined for L-histidine (**1**), L-histidinol (**2**), 4-hydroxymethylimidazole (**3**), histidylglycine (**4**), glycylhistidine (**5**), and histidylalanine (**6**). Each of compounds **1–6** and $NAD^+$ were incubated in the presence of NADase and the respective incubation mixtures were checked for the formation of possible ADP-ribosylated product by thin layer chromatography (TLC), and then the product was isolated by column chromatography on DEAE-Sephadex A-25.

A product in 45% yield† from the incubation with **1** exhibited an intense ($M^+ - 1$) ion peak at *m/z* 695 in the negative FAB-MS spectrum, and showed, in the $^1H$ NMR spectrum, two anomeric protons (δ 5.63 and 6.08) as well as ten non-exchangeable protons (δ 4.12–4.41) of two riboses together with five non-exchangeable protons (δ 3.02, 3.17, 3.92, 7.27 and 7.99) of histidine and two adenine-ring protons (δ 8.19 and 8.45), demonstrating that the product was ADP-ribosylated L-histidine (**7**).



**9**

**7** $R^1$=H ; $R^2$=$CO_2H$
**8** $R^1$=H ; $R^2$=$CH_2OH$
**10** $R^1$=H ; $R^2$=$CONHCH_2CO_2H$
**11** $R^1$=$COCH_2NH_2$ ; $R^2$=$CO_2H$
**12** $R^1$=H ; $R^2$=$CONHCH(CH_3)CO_2H$

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† Based on NAD used.

This reaction occurred irrespective of the configuration (L- or D-form) of histidine. Similar ADP-ribosylation was observed with **3**, and an analogous dinucleotide **9** was isolated in 35% yield. Compound **2** seemed also to undergo the enzymatic ADP-ribosylation judging from TLC analysis, but virtually none of the desired product **8** could be isolated; this is presumably due to its instability during the isolation processes. In the case of respective reactions with dipeptides **4** and **5**, corresponding new dinucleotides were formed and successfully isolated in 31–35% yield. They exhibited satisfactory mass and $^1$H NMR spectral properties compatible with the structure of **10** and **11**, respectively: in both cases, MS spectra showed *m/z* 752 ($M^+ - 1$) and $^1$H NMR spectra two doublets (δ 5.96 and 6.20) characteristic of two anomeric protons of the dinucleotides. In addition, dipeptide **6** was also found to undergo the enzymatic reaction, giving the ADP-ribosylated product **12** in 24% yield.

The ribosylation site in imidazole moiety of dinucleotides thus obtained was presumed to be exclusively the $N^1$-position, but not the $N^3$-position, on the basis of $^1$H NMR spectral findings: the imidazole ring at the 5- as well as the 2-position showed lower-field resonances (0.24–0.82 ppm) in all cases as compared with those in the corresponding substrate bases themselves. These are compatible with previous observations[4] that an N-atom bearing an adjacent bulky substituent does not readily undergo this enzymatic ADP-ribosylation. Thus, it has been shown here that the ADP-ribosylation to histidine and related dipeptides occurs by ADP-ribosyl transferase-like activity of porcine-brain NADase.

Recently 'NADase' has been noted in connection with its function associated with cellular signal transduction: new NADase activity catalysing, besides the normal hydrolysis of NAD to ADP-ribose and nicotinamide, the reversible conversion of NAD into cyclic ADP-ribose, was discovered in cell-surface CD38 antigen on human leukocytes[6] and the cyclic NAD metabolite is suggested to play an important role in intracellular $Ca^{2+}$ homeostasis.[7] Although it is as yet unclear whether the tissue-derived NADase has a similar function as the leukocyte-derived NADase and why the NADase exhibits non-specific ADP-ribosyltransferase-like activity, it is noteworthy that porcine-brain NADase catalyses trans-ADP-ribosylation from NAD to histidine and related dipeptides, as composition units of protein, as well.

## Experimental

*General.* FAB-MS (negative) spectra were determined on a JEOL JMX-DX 300 instrument. The $^1$H NMR spectra were recorded in $D_2O$ on a Bruker MSL-400 spectrometer (400 MHZ). Thin layer chromatography (TLC) was performed on silica gel $60F_{254}$ HPTLC plates (Merck). Column chromatography was carried out on DEAE-Sephadex A-25 and monitored by means of LKB Uvicord II (254 nm). Aqueous tris(hydroxymethyl)aminomethane–hydrochloric acid buffer solution (Tris–HCl/pH 7.2) was used as the incubation system. NAD and dipeptides were obtained from Sigma, and L- and D-histidines, L-histidinol and 4-(hydroxymethyl)imidazole were from Aldrich.

*Porcine-brain NADase [EC 3.2.2.5].* The crude particulate enzyme was prepared from fresh porcine brain by the method of Zatman *et al.*[8] The colloidal homogenate containing ca. 0.4 U‡ per ml of NADase activity was used without further purification.

*L-Histidine adenine dinucleotide (7)* β-NAD (0.96 g, 1.4 mmol) and L-histidine (0.82 g, 5.3 mmol) were incubated with NADase (15 ml, 6 U) in 0.2 M Tris–HCl (60 ml, pH 7.2) at 37°C for 26 h. NAD disappeared at that point of incubation time. The reaction mixture was worked up in a similar manner as described previously.[4] The crude mass (0.85 g) thus obtained was dissolved in water (40 ml) and applied to a column of DEAE-Sephadex A-25 ($HCO_3^-$ form). The column was washed with 0.8% (w/w) aqueous $NH_4HCO_3$ solution and then eluted with a 4% solution of the same salt. The first eluted major component was ADP-ribose and the second major component was the desired product. The corresponding eluate fractions were collected and evaporated to dryness *in vacuo* to give a solid mass. The isolated mass was subjected to further chromatography and appropriate fractions were repeatedly lyophilized to give **7** (432 mg, 45% yield) as a pale yellow ammonium salt. An analytical sample was obtained by further drying over $P_2O_5$ *in vacuo* at 40°C for 12 h. MS: *m/z* 695 ($M^+ - 1$). $^1$H NMR ($D_2O$): δ 3.02 (1 H, dd, *J* 8.8 and 15.4 Hz), 3.17 (1 H, dd, *J* 4.2 and 15.4 Hz), 3.92 (1 H, dd, *J* 4.2 and 8.8 Hz), 4.12 (2 H, br s), 4.22 (3 H, br), 4.36 (3 H, br), 4.41 (1 H, t, *J* 5.0 Hz), 4.51 (1 H, dd, *J* 4.0 and 5.0 Hz), 5.63 (1 H, d, *J* 6.0 Hz), 6.08 (1 H, d, *J* 5.9 Hz), 7.27 (1 H, s), 7.99 (1 H, s), 8.19 (1 H, s), 8.45 (1 H, s). Anal. $C_{21}H_{30}N_8O_{15}P_2 \cdot 2NH_3 \cdot 2H_2O$: C, H, N, P.

*4-Hydroxymethylimidazole adenine dinucleotide (9)* A mixture of 4-hydroxymethylimidazole (376 mg, 3.8 mmol) and NAD (725 mg, 1.1 mmol) was incubated with NADase (15 ml, 6 U) in Tris–HCl (50 ml) at 37°C for 20 h. The incubation mixture was treated in a similar manner as described above to give **9** (254 mg, 35% yield) as the ammonium salt. MS: *m/z* 638 ($M^+ - 1$). $^1$H NMR ($D_2O$): δ 1.91 (2 H, s), 4.11 (2 H, m), 4.24 (3 H, br), 4.37 (2 H, br q), 4.45 (1 H, t, *J* 5.0), 4.50 (1 H, dd, *J* 4.0 and 5.0 Hz), 4.72 (1 H, t, *J* 5.0 Hz), 5.62 (1 H, d, *J* 6.1 Hz), 6.08 (1 H, d, *J* 5.9 Hz), 7.24 (1 H, s), 7.77 (1 H, s), 8.20 (1 H, s), 8.45 (1 H, s). Anal. $C_{19}H_{27}N_7O_{14}P_2 \cdot 2NH_3 \cdot 2H_2O$: C, H, N, P.

‡ U is the activity of NADase which will cleave 1 μmol of NAD per min.

*Histidylglycine adenine dinucleotide (***10***)* A mixture of histidylglycine (82 mg, 0.39 mmol) and NAD (74 mg, 0.11 mmol) was incubated with NADase (5 ml, 2 U) in Tris–HCl (30 ml) for 15 h. The incubation mixture was treated in a similar manner as described above to provide **10** (26 mg, 31% yield) as the ammonium salt. MS: *m/z* 752 ($M^+ - 1$). $^1$H NMR ($D_2O$): δ 4.23 (3 H, br), 4.3–4.4 (6 H, br), 4.45 (3 H, br), 4.59 (2 H, dd, *J* 2.8 and 6.0 Hz), 5.95 (1 H, dd, *J* 2.8 and 6.0 Hz), 5.97 (1 H, d, *J* 6.0 Hz), 6.20 (1 H, d, *J* 5.8 Hz), 7.95 (1 H, s), 8.08 (1 H, s), 8.26 (1 H, s), 8.33 (1 H, s). Anal. $C_{23}H_{33}N_9O_{16}P_2 \cdot 2NH_3 \cdot 2H_2O$: C, H, N, P.

*Glycylhistidine adenine dinucleotide (***11***).* Glycylhistidine (348 mg, 1.6 mmol) and NAD (376 mg, 0.56 mmol) were incubated with NADase (10 ml, 4 U) for 20 h. The resulting mixture was treated in the manner described above to provide **11** (103 mg, 27% yield) as the ammonium salt. MS: *m/z* 752 ($M^+ - 1$). $^1$H NMR ($D_2O$): δ 4.18–4.26 (3 H, br), 4.28–4.40 (6 H, br), 4.45 (3 H, br), 4.59 (2 H, dd, *J* 2.8 and 6.0 Hz), 5.94 (1 H, dd, *J* 2.8 and 6.0 Hz), 5.98 (1 H, d, *J* 6.0 Hz), 6.20 (1 H, d, *J* 5.8 Hz), 7.95 (1 H, s), 8.08 (1 H, s), 8.26 (1 H, s), 8.34 (1 H, s). Anal. $C_{23}H_{33}N_9O_{16}P_2 \cdot 2NH_3 \cdot 2H_2O$: C, H, N, P.

*Histidylalanine adenine dinucleotide (***12***)* A mixture of histidylalanine (80 mg, 0.35 mmol) and NAD (80 mg, 0.12 mmol) was incubated with NADase (5 ml, 2 U) for 16 h. The incubation mixture was treated as described above to give **12** (19 mg, 24% yield) as the ammonium salt. MS: *m/z* 766 ($M^+ - 1$). $^1$H NMR ($D_2O$): δ 1.34 (3 H, d, *J* 7.3 Hz), 4.20–4.26 (3 H, br), 4.28–4.40 (6 H, br), 4.45 (3 H, br), 4.58 (2 H, dd, *J* 3.0 and 7.4 Hz), 5.95 (1 H, dd, *J* 3.0 and 6.0 Hz), 5.98 (1 H, d, *J* 6.0 Hz), 6.20 (1 H, d, *J* 5.8 Hz), 7.94 (1 H, s), 8.05 (1 H, s), 8.24 (1 H, s), 8.32 (1 H, s). Anal. $C_{24}H_{35}N_9O_{16}P_2 \cdot 2NH_3 \cdot 2H_2O$: C, H, N, P.

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## References

1. Anderson, B. M. and Kaplan, N. O. *J. Biol. Chem. 234* (1959) 1226.
2. Walter, P. and Kaplan, N. O. *J. Biol. Chem. 238* (1963) 2823.
3. Tono-oka, S., Tone, Y., Marquez, V. E., Cooney, D. A., Sekikawa, I. and Azuma, I. *Bull. Chem. Soc. Jpn. 58* (1985) 309.
4. Tono-oka, S. and Azuma, I. *Gazz. Chim. Ital. 121* (1991) 297.
5. Tanuma, S., Kawashima, K. and Endo, H. *J. Biol. Chem. 263* (1988) 5485.
6. Howard, M., Grimaldi, J. C., Bazan, J. F., Lund, F. E., Santos-Argumedo, L., Parkhouse, R. M. E., Walseth, T. F. and Lee, H. C. *Science 262* (1993) 1056.
7. Kim, H., Jacobson, E. L. and Jacobson, M. K. *Science 261* (1993) 1330.
8. Zatman, L. J., Kaplan, N. O., Colowick, S. P. and Ciotti, M. M. *J. Biol. Chem. 209* (1954) 467.

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