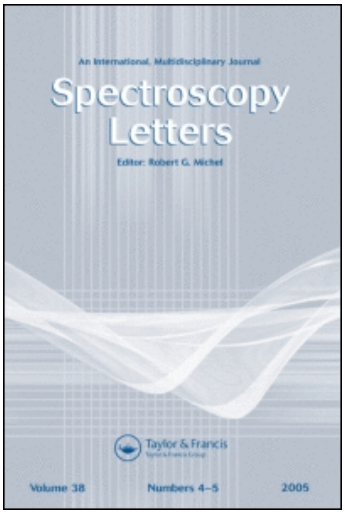

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

STUDIES ON COPPER-CONTAINING NAD GLYCOHYDROLASE FROM *AGKISTRODON ACUTUS* VENOM BY FLUORESCENCE AND CD SPECTROSCOPY

Shuang Ding Wu[a]; Zhengang Zhu[a]; Yanli Liu[a]
[a] Laboratory of Internal Friction and Defects in Solids, Institute of Solid State Physics, Chinese Academy of Science, HeFei, Anhui, People's Republic of China

Online publication date: 14 August 2002

To cite this Article Wu, Shuang Ding , Zhu, Zhengang and Liu, Yanli(2002) 'STUDIES ON COPPER-CONTAINING NAD GLYCOHYDROLASE FROM *AGKISTRODON ACUTUS* VENOM BY FLUORESCENCE AND CD SPECTROSCOPY', Spectroscopy Letters, 35: 4, 517 — 522
To link to this Article: DOI: 10.1081/SL-120013887
URL: http://dx.doi.org/10.1081/SL-120013887

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

SPECTROSCOPY LETTERS, 35(4), 517–522 (2002)

STUDIES ON COPPER-CONTAINING NAD GLYCOHYDROLASE FROM *AGKISTRODON ACUTUS* VENOM BY FLUORESCENCE AND CD SPECTROSCOPY

Shuang Ding Wu,* Zhengang Zhu, and Yanli Liu

Laboratory of Internal Friction and Defects in Solids,
Institute of Solid State Physics, Chinese
Academy of Science, HeFei, Anhui 230031,
People's Republic of China

ABSTRACT

The properties of NAD Glycohydrolase (NADase), purified from *Agkistrodon acutus* venom, have been studied by fluorescence and CD spectroscopy. The fluorescence intensities of NADase decrease by about 1% or 3% when the concentrations of I^- ion are 0.1 mol/L or 0.2 mol/L in the NADase solutions, respectively. However, the fluorescence intensities of the NADase are quenched by about 25% and 48%, respectively, with further addition of 1 mmol/L EDTA into solutions. CD spectra also suggested that EDTA could remove Cu^{2+} ion from NADase molecule and the conformation

*Corresponding author. Fax: + 86-551-5591434; E-mail: sdwu@mail.issp.ac.cn

of NADase changed much. So Cu^{2+} ion is very important to maintain the geometrical structure of NADase.

Key Words: NADase; Cu^{2+} ion; Fluorescence; CD; Spectroscopy

INTRODUCTION

NAD glycohydrolase (NADase, EC 3.2.2.5) can catalyze the hydrolysis of the nicotinamide-ribose bond to form nicotinamide and adenosine diphosphoribose (ADP-Rib). NADase is present in various microorganisms,[1–7] animal tissues[8–13] and animal venom.[14–17] In mammals, NADases are found in association with the membranes of most cell types, including erythrocytes.[18–21]

Snake venom contains many kinds of proteins and enzymes. The presence of NADase in snake venom was first demonstrated by Tatsuki et al.[14] NADase was first isolated from Chinese *Agkistrodon acutus* venom by Huang et al. and the properties of NADase were studied to demonstrate that it is a copper-containing protein.[17] However, the property of NADase was not further investigated. In this letter, we report the results on NADase purified from *Agkistrodon acutus* by fluorescence and CD spectroscopy. It was found that Cu^{2+} ion is very important to maintain the geometrical structure of NADase.

EXPERIMENTAL

NADase was purified to electrophoretic homogeneity according to the improved method (Data not shown). Other reagents were of high grade commercially available and were used without further purification. Protein concentration was determined by the method of Bradford[22] with bovine serum albumin as a standard.

Fluorescence spectra were performed with Shimadzu RF-5000 spectrofluorimeter. NADase (0.02 mg/mL) were dissolved in 0.02 mol/L Tris-HCl buffer solutions containing 0.1 mol/L NaCl. The KI reagent and KI+EDTA reagent were added into the series of NADase solutions and their final concentrations were 0.1 mol/L KI, 0.2 mol/L KI, 0.1 mol/L KI+1 mmol/L EDTA and 0.1 mol/L KI+1 mmol/L EDTA, respectively.

CD spectrum was determined by a JASCO 500C spectropolarimeter from 190 nm to 250 nm. One sample contained 0.1 mg/mL NADase in 0.02 mol/L Tris-HCl (pH 7.4) buffer solution. The other sample contained

0.1 mg/mL NADase and 1 mmol/L EDTA in 0.02 mol/L Tris-HCl (pH 7.4) buffer solution.

RESULTS AND DISCUSSION

Figure 1 shows fluorescence spectra of the NADase, excited at 281.6 nm, measured in various solutions containing different concentration of I^- ion or EDTA. When 0.1 mol/L I^- ion is added into the enzyme solution, the fluorescence intensity decreases only a little and equals to about 99% relative to the original enzyme. Increasing the concentration of the added I^- ion to 0.2 mol/L, the fluorescence intensity changes slightly and is about 97% of the original fluorescence intensity. However, if 1 mmol/L EDTA is added into the solution mentioned above, the quenching fluorescence ability of I^- ion increases greatly and their fluorescence intensities are 75% and 52% relative to the original enzyme fluorescence, respectively. This result shows that the Trp residues of NADase are hidden deeply in the NADase molecules. I^- ion can not

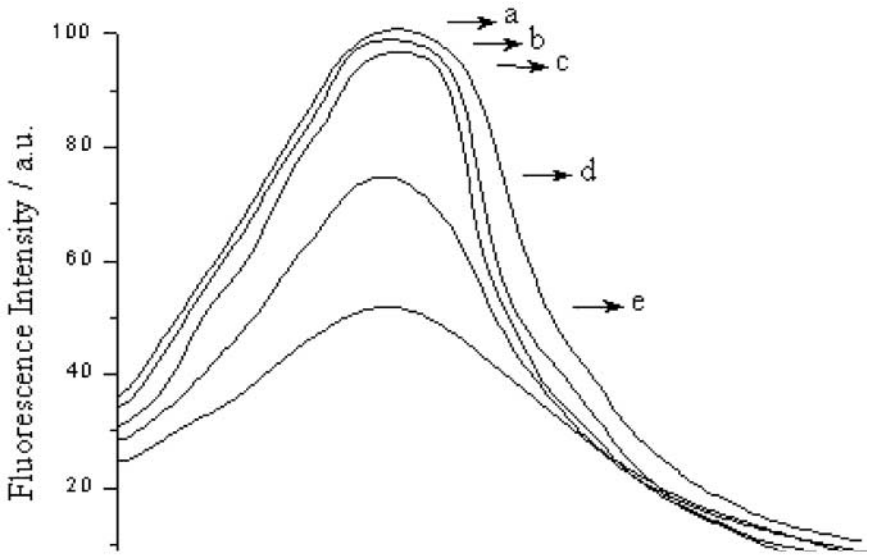

Figure 1. Fluorescence quenching of NADase by I^- ion. NADase concentration: 0.02 mg/mL, 0.02 mol/L Tris-HCl buffer solution (pH7.4) containing 0.1 mol/L NaCl. a, 0 mol/L KI; b, 0.1 mol/L KI; c, 0.2 mol/L KI; d, 0.1 mol/L KI+1 mmol/L EDTA; e, 0.2 mol/L KI+1 mmol/L EDTA. Excitation wavelength: 281.6 nm.

approach the Trp residues, so the I^- ion can't quench the fluorescence efficiently. However, EDTA is added into NADase solution and removes Cu^{2+} ion from NADase molecules, which results that the geometric structure of NADase changes greatly and Trp residues are relatively exposed out. I^- ion can approach and interact with Trp residues efficiently, which leads to its fluorescence quenching. Based on these results, Cu^{2+} ion is inferred to be important to maintain the geometric structure of NADase.

In order to further investigate the role of Cu^{2+} ion in NADase, the CD spectra was measured. CD signals in the far-UV region arise mainly from the secondary structure of the protein backbone and are very sensitive to all conformations. As shown in Fig. 2, the CD spectrum of NADase change very much. The CD spectrum of native NADase displays one negative maximum at about 206 nm and a shoulder at about 220 nm. When 1 mmol/L EDTA was added into the NADase solution, the maximum at 206 nm decreased much, and the shoulder at 220 nm nearly disappeared. These results suggested the conformation of NADase may change very much by the treatment. EDTA is a ligand with stronger chelation and can remove Cu^{2+} ions from NADase molecules. When Cu^{2+} ion was removed

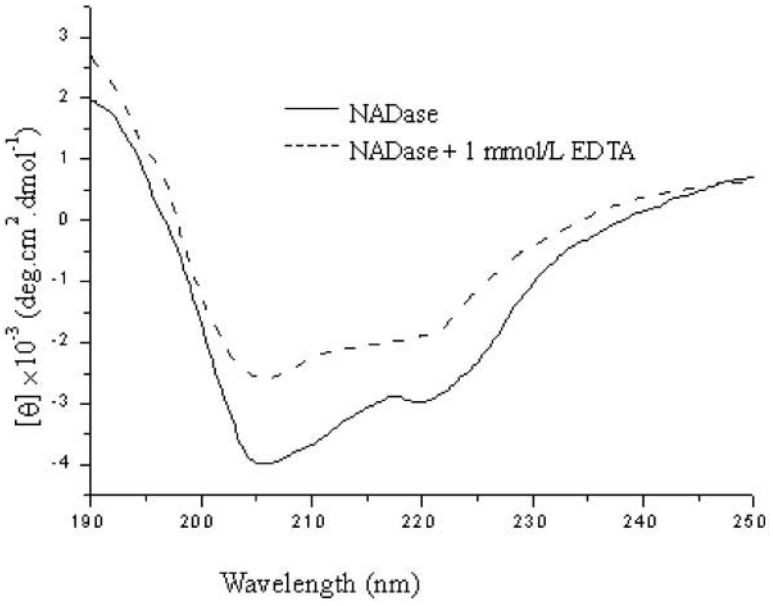

Figure 2. CD spectra of NADase in 0.02 mol/L Tris-HCl buffer solution (pH7.4) containing 0.1 mol/L NaCl. NADase concentration: 0.01 mg/mL.

from NADase molecule by added EDTA, the second structure of NADase changed, which demonstrated that Cu^{2+} ion is very important to maintain the conformation of NADase. It is consistent with the results of fluorescence spectroscopy.

In summary, fluorescence and CD spectrum on the NADase have been used to study the role of Cu^{2+} in the NADase enzyme. It was found that Cu^{2+} ion is very important to maintain the conformation of NADase. The removal of Cu^{2+} from NADase molecule resulted that the geometric structure was partially unfolded. The role of Cu^{2+} in NADase molecule will be further investigated.

REFERENCES

1. Gerlach, D.; Ozegowski, J.H.; Gunther, E.; Vettermann, S.; Kohler, W. FEMS Microbiol. Lett. **1996**, *136*, 71–78.
2. Gerlach, D.; Ozegowski, J.H.; Gunther, E.; Vettermann, S.; Kohler, W. Adv. Exp. Med. Biol. **1997**, *418*, 601–603.
3. Everse, K.E.; Everse, J.; Simeral, L.S. Methods Enzymol. **1980**, *66*, 137–44.
4. Cho, Y.S.; Han, M.K.; Kwark, O.S.; Phoe, M.S.; Cha, Y.S.; An, N.H.; Kim, U.H. Comp. Biochem. Physiol. B Biochem. Mol. Biol. **1998**, *120*, 175–181.
5. Pace, M.; Agnellini, D.; Lippoli, G.; Berger, R.L. Adv. Exp. Med. Biol. **1997**, *419*, 389–397.
6. Karasawa, T.; Takasawa, S.; Yamakawa, K.; Yonekura, H.; Okamoto, H.; Nakamura, S. FEMS Microbiol. Lett. **1995**, *130*, 201–204.
7. Ajdic, D.; McShan, W.M.; Savic, D.J.; Gerlach, D.; Ferretti, J.J. FEMS Microbiol. Lett. **2000**, *191*, 235–241.
8. Muller-Steffner, H.; Schenherr-Gusse, I.; Tarnus, C.; Schuber, F. Arch. Biochem. Biophys. **1993**, *304*, 154–162.
9. Ziegler, M.; Jorcke, D.; Zhang, J.; Schneider, R.; Klocker, H.; Auer, B.; Schweiger, M. Biochemistry **1996**, *35*, 5207–5212.
10. Jorcke, D.; Ziegler, M.; Schweiger, M. Adv. Exp. Med. Biol. **1997**, *419*, 447–451.
11. Zhang, J.; Ziegler, M.; Schneider, R.; Klocker, H.; Auer, B.; Schweiger, M. FEBS Lett. **1995**, *377*, 530–534.
12. Okayama, H.; Ueda, K.; Hayaishi, O. Methods Enzymol. **1980**, *66*, 151–154.
13. Orsomando, G.; Polzonetti, V.; Natalini, P. Comp. Biochem. Physiol. B Biochem. Mol. Biol. **2000**, *126*, 89–98.

14. Katsuki, T.; Iwanaga, S.; Oshima, G.; Suzuki, T. Toxicon **1975**, *13*, 211–220.
15. Yost, D.A.; Anderson, B.M. J. Biol. Chem. **1981**, *256*, 3647–3653.
16. Anderson, B.M.; Yost, D.A.; Anderson, C.D. Methods Enzymol. **1986**, *122*, 173–181.
17. Huang, W.Z.; Wang, C.; Luo, L.Q.; Lu, Z.X. Toxicon **1988**, *26*, 535–542.
18. Kim, U.H.; Kim, M.K.; Kim, J.S.; Han, M.K.; Park, B.H.; Kim, H.R. Arch Biochem Biophys. **1993**, *305*, 147–152.
19. Goodman, S.I.; Wyatt, R.J.; Trepel, J.B.; Neckers, L.M. Comp Biochem Physiol B. **1982**, *71*, 333–336.
20. Saxty, B. A.; van Heyningen, S. Biochem. J. **1995**, *310* (Pt 3), 931–937.
21. Berthelier, V.; Tixier, J.M.; Muller-Steffner, H.; Schuber, F.; Deterre, P. Biochem. J. **1998**, *330* (Pt 3), 383–1390.
22. Bradford, M.M. Anal. Biohem. **1976**, *72*, 248–254.

Received January 13, 2002
Accepted April 30, 2002