SPECIFIC MODIFICATION OF NAD BINDING SITE OF CHICKEN LIVER XANTHINE DEHYDROGENASE WITH 5'-$_p$-FLUOROSULFONYLBENZOYLADENOSINE.

Tomoko Nishino, Takeshi Nishino and Keizo Tsushima
Department of Biochemistry, Yokohama City University School of Medicine, Yokohama 232, Japan

Introduction

Conversion of dehydrogenase (D-form) to oxidase form (O-form) is known to occur in rat, but not in chicken liver xanthine dehydrogenase. To clarify the structural difference of active sites around FAD domain between rat and chicken enzymes, chemical modification by 5'-Fluorosulfonylbenzoyladenosine (5'-FSBA), a NAD analogue, has been carried out (1,2,3.). In this paper 5'-FSBA was found to be very specific modifier of NAD binding sites of both enzymes. Modified amino acid residue of chicken enzyme by 5'-FSBA was identified as tyrosine.

Materials and Methods

Xanthine dehydrogenase was purified from livers of chicken fed a high protein diet by the procedure of Nishino (4). 5'-FSBA, N-acetyltyrosine were obtained from Sigma, ^{14}C-FSBA from New England Nuclear and all other chemicals were of analytical grade. Cellulose TLC plate from Eastman. The enzyme was assayed by measuring changes of absorbance at 340 nm under standard conditions (4). Inactivation reaction was performed at pH 7.8, 25°C with various concentrations of FSBA. Enzyme concentration was 2.5×10^{-6}M. Experiments of ^{14}C-FSBA incorporation was performed at 0°C. After various incubation periods, residual enzyme activities were checked and reactions

Flavins and Flavoproteins

were terminated by addition of ethanol (final 50%) and precipitates were washed thoroughly and radioactivities were counted by sintillation counter.
Carboxybenzenesulfonyl-tyrosine (CBS-Tyr) was synthesized by the procedure of Colman et al. (2). FSBA inactivated enzyme was hydrolysed for 20 hrs with 6N HCl at 110°C. Hydrolysate and synthesized CBS-Tyr were subjected first to thin layer electrophoresis in the horizontal direction at pH 6.3 in pyridine- acetate buffer. After drying the plate it was subjected to chromatograpy in butanol: acetic acid: water/ 4:1:5 in vertical direction (2). Amino acids and CBS-Tyr on TLC plate were detected by using ninhydrin reaction.

Results and Discussions

Fig. 1 shows time course of inactivation of xanthine dehydrogenase with various concentrations of 5'-FSBA. The reaction displayed pseudo-first order kinetics. In inset of Fig. 1, the double reciplocal plots of inactivation velocity versus 5'-FSBA concentration was extrapolated to origin. The results indicate that enzyme and 5'-FSBA do not form a complex reversibly prior to the inactivation process. As shown in Fig. 2, 1 mol of ^{14}C-FSBA per mol of FAD was incorporated into chicken enzyme for complete inactivation. Inactivation of xanthine dehydrogenase was completely protected by NAD (data was not shown). Such a stoichiometry of ^{14}C-FSBA incorporation and a protection by NAD indicated that 5'FSBA reacted with amino acid residue of NAD binding site specifically. 5'-FSBA inactivated xanthine dehydrogenase of rat and chicken liver were apparently very slowly and slightly reactivated by DTT, because the inactivation of both enzymes was caused by the reaction with DTT. Fig. 3 showed the isolation of CBS- Tyr from hydrolysates of 5'-FSBA modified chicken enzyme. Identification of CBS-Try was made by comparison with authentic sample by the method of Colman et al.

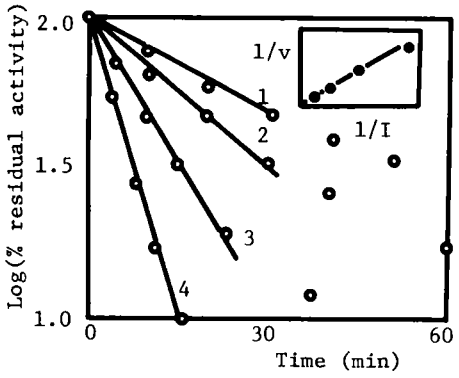

Fig. 1. Time course of inactivation of chicken xanthine dehydrogenase by 5'-FSBA. 5'-FSBA concentrations are, 1; 27.4μM, 2; 50.9μM, 3; 101.8μM, 4; 208.5μM, respectively.

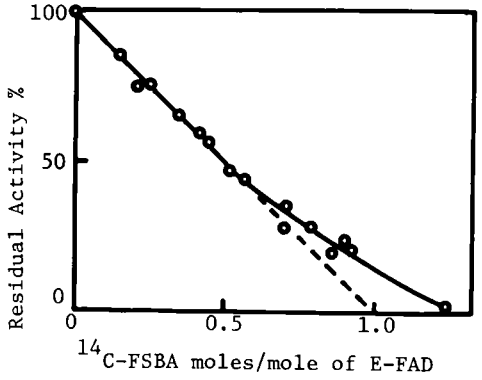

Fig. 2. Incorporation of ^{14}C-FSBA to chicken xanthine dehydrogenase. After reaction with ^{14}C-FSBA for various incubation periods at pH 7.8 at 0°C, residual activities were determined and quenched by ethanol, then radioactivities were counted.

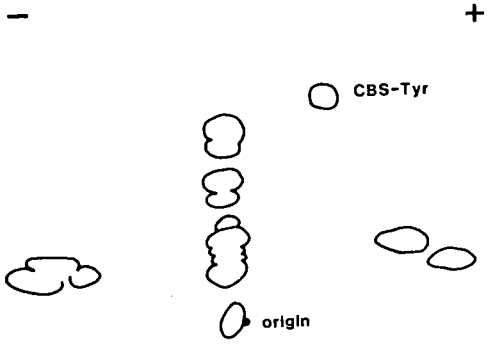

Fig. 3. Identification of modified amino acid. 5'-FSBA modified xanthine dehydrogenase was hydrolyzed and the sample was subjected first to thin layer electrophoresis in the horizontal direction and secondary to chromatography in vertical direction.

Inactivation of chicken and rat xanthine dehydrogenase by butanedione implicated active arginine residue in NAD binding site (unpublished data). Rat xanthine dehydrogenase inactivated by butanedione could be converted to O-form by SH-modifier, suggesting that NAD binding site is not concerned in D-O conversion. Furthermore, 5'-FSBA also inactivated rat liver xanthine dehydrogenase in a similar proportion to chicken enzyme. 5'-FSBA inactivated rat liver xanthine dehydrogenase could not be converted to O-form by SH-modifier treatment. In chicken enzyme, tyrosine was identified as an active amino acid residue at NAD binding site. These results also support that a different site from NAD binding site is concerned in O-D interconversion reaction.

Acknowledgement

This work was supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science and Culture (No. 58480142)

References

1. Esch, S. F., Allison, S. W.: J. Biol. Chem. 253, 6100-6106 (1978)
2. Saradambal, V. K., Bednar, A. R., Colman, F. R.: J. Biol. Chem. 256, 11866-11872 (1981).
3. Colman, F. R.: Ann. Rev. Biochem. 52, 76-91 (1983).
4. Nishino T.: Biochem. Biophys. Acta 341, 93-98 (1974).