UNIVERSITY OF CALIFORNIA

San Diego

Purification and Properties of Aldehyde Dehydrogenase from *Clostridium kluyveri*

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Chemistry

by

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1977

The dissertation of Linda Tombras Smith is approved, and it is acceptable in quality and form for publication on microfilm:

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1977

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ACKNOWLEDGMENTS

I would like to thank Dr. Nathan O. Kaplan for his scientific guidance, support, and encouragement. Dr. G. D. Novelli is gratefully acknowledged for providing the Clostridium kluyveri cells. Thanks also to Dr. J. Everse for assistance with the ultracentrifugation and stopped-flow studies. Drs. G. M. Smith and C. Y. Lee ran and assisted in the interpretation of the nuclear magnetic resonance spectra. Thanks to Dr. Y. Levin for providing his expertise in the field of affinity chromatography. Drs. L. J. Arnold and K. S. You are acknowledged for their helpful advice.

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PUBLICATIONS

"Purification and Characterization of Aldehyde Dehydrogenase from Clostridium kluyveri." Fed. Proc., 35: 1750 (1976).

FIELD OF STUDY

Major Field: Biochemistry

Studies in Enzymology
Professor Nathan O. Kaplan

ABSTRACT OF THE DISSERTATION

Purification and Properties of Aldehyde Dehydrogenase

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Professor Nathan O. Kaplan, Chairman

Coenzyme A-linked aldehyde dehydrogenase from Clostridium kluyveri catalyzes the oxidation of acetaldehyde in the presence of CoA and oxidized pyridine nucleotide to form acetyl CoA and reduced pyridine nucleotide. The enzyme was purified approximately 90 fold over crude extracts to a specific activity of 50 units/mg protein. From polyacrylamide gel electrophoresis, the enzyme preparation was estimated to be 35% pure. Although the dehydrogenase showed some affinity toward substrate and hydrophobic ligands bound to Sepharose, none of the gels tested were found to be useful for purification purposes. The enzyme was partially stabilized by thiols at low temperatures.

From active enzyme centrifugation studies, aldehyde dehydrogenase was found to have a sedimentation coefficient of $S_{20,w} = 7.4$. From gel filtration studies, the Stokes radius of the enzyme in the presence of substrates was found to be 9.5 nm and in the absence of substrates was 11.0 nm. Using the values found for the sedimentation coefficient and the Stokes radius, the molecular weight of the enzyme in the presence of substrates was calculated to be 290,000 daltons. The frictional ratio was 2.2.

Aldehyde dehydrogenase can utilize both NAD^+ and $NADP^+$ to catalyze the formation of acetyl CoA. The rate obtained with $NADP^+$ was about 30% of that obtained with NAD^+. To a limited extent, the dehydrogenase may utilize thiols other than CoA as acetyl acceptors. A number of methods were employed in order to exclude the possibility that these thiols act merely by recycling trace amounts of CoA that might be in the enzyme preparation via nonenzymatic transacetylation.

Initial velocity and product inhibition studies were conducted in order to elucidate the order of substrate binding and product release. A Bi Uni Uni Uni Ping Pong kinetic mechanism was proposed in which NAD^+ binds to the enzyme first, acetaldehyde second, and NADH is released. Then CoA binds and acetyl CoA is released. At K_M levels of NAD^+ and CoA, substrate inhibition by CoA was observed. It was suggested that CoA binds to free enzyme to form a dead-end E·CoA inhibition complex.

Phosphotransacetylase from Clostridium kluyveri was purified more than 140 fold over crude extracts. by a combination of ammonium sulfate fractionation and affinity chromatography with C^8-(aminohexyl)-amino-desulfo-CoA bound to Sepharose. The purified protein was judged to be over 70% pure by polyacrylamide gel electrophoresis.