

AMINO ACID SEQUENCE OF A COFACTOR PEPTIDE FROM TRIMETHYLAMINE DEHYDROGENASE

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1. Introduction

Trimethylamide dehydrogenase (EC 1.5.99.7), from facultative methylotropic bacteria, catalyzes the oxidative demethylation of trimethylamine to dimethylamine and formaldehyde [1]. This enzyme has mol. wt 147 000 [1] and contains 4 g-atoms each of non-heme Fe and labile S, as well as a covalently-bound yellow coenzyme [2]. The structure of the Fe-S center in the enzyme has recently been established to be a tetrameric Fe_4S_4 core unit [3].

This enzyme is of uncommon interest from several standpoints. First, it contains a novel coenzyme, which recent investigations indicate to be a flavin mononucleotide derivative with a cysteine substituted in the 6 position by way of a thioether linkage [14,15]. Thus, trimethylamine dehydrogenase is the first enzyme discovered to contain covalently bound flavin with substitution in other than the 8 α position [4].

In addition to this novel cofactor, this enzyme is of interest in that typical 'suicide' inhibitors of mitochondrial monoamine oxidase, such as cyclopropylamines and substituted hydrazines, are also potent inhibitors of trimethylamine dehydrogenase, although it does not oxidize monoamines [5]. Another unusual

feature of the enzyme is that on addition of substrate to the enzyme a complex pattern of EPR signals is obtained, indicative of a unique spin-coupled interaction between the organic chromophore and an Fe-S center and that the formation of this complex appears to be the rate-limiting step in catalysis [6].

As a first step in establishing the structural bases of several of these properties of trimethylamine dehydrogenase, the present paper described the primary structure at the active center containing the coenzyme. This was found to be:

Cys(Flavin)-Ile-Gly-Ala-Gly-Ser-Asp-Lys-
Pro-Gly-Phe-Gln

2. Materials and methods

Trimethylamine dehydrogenase was obtained from bacterium W3A1 and purified as reported [1]. The peptide-bound coenzyme was obtained by a series of steps involving trichloroacetic acid treatment, trypsin-chymotrypsin digestion, and column chromatography on Florisil, DEAE-cellulose, and phosphocellulose [14]. Acid hydrolysis was carried out in vacuo for 16 h at 105°C in 6 N HCl containing 4% thioglycolic acid [7]. Amino acid analyses were performed on a modified Hitachi Perkin-Elmer 034 Liquid Chromatograph.

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The cofactor peptide (1 mM) was incubated at 38°C for 24 h with aminopeptidase M (1 mg/ml) in 0.2 M *N*-ethylmorpholinium acetate, pH 8.0. This peptide was also incubated under the same conditions with carboxypeptidase A (0.2 mg/ml).

A smaller cofactor peptide was obtained by digestion of the tryptic-chymotryptic peptide (1 mM) with nagarase (0.2 mg/ml in 0.1 M ammonium bicarbonate, pH 9.0, 16 h, 37°C). This peptide was purified on phosphocellulose (pyridinium form) equilibrated with 5% (v/v) formic acid.

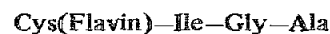
The nagarase peptide was sequenced by the manual dansyl-Edman technique [8] and the tryptic-chymotryptic peptide was sequenced on a Beckman Model 890C Sequencer. Acetylated cytochrome *c* (2 mg) was included to minimize extraction losses [9]. The phenylthiohydantoin derivatives of the amino acids were identified by two-dimensional thin-layer chromatography and gas-liquid chromatography, in some cases after trimethylsilylation [10,11].

3. Results and discussion

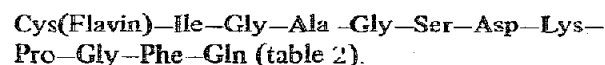
The amino acids obtained after acid hydrolysis and aminopeptidase M digestion of the tryptic-chymotryptic peptide and the nagarase peptide derived there-

from, both of which contain the cofactor, are listed in table 1. The low values for glycine and lysine after aminopeptidase M digestion may be due to their linkage to proline.

The amino acid sequence of the nagarase peptide was found to be:



and for the tryptic-chymotryptic peptide:



Since all amino acids detected by acid hydrolysis can be assigned a position in the peptide sequence of each peptide, the amino acyl residue released in step 1 of the sequencing procedure must be the residue containing the covalently bound cofactor, i.e., cysteinyl flavin. That an additional amino acid must be present is clear from the following considerations. First, studies on the isolated amino acyl cofactor, obtained by aminopeptidase M digestion of the peptide [14] indicated that the amino acyl-cofactor linkage is not cleaved to a significant extent by either acid hydrolysis or aminopeptidase M digestion. Moreover, the Edman degradation does not result in its detection and

Table 1
Amino acid composition of cofactor peptides from trimethylamine dehydrogenase^a

Amino acid	Tryptic-chymotryptic peptide			After nagarase treatment
	Acid hydrolysis	Aminopeptidase M digestion	Nearest integer	Acid hydrolysis
Asp	1.11	0.90	1	
Ser	0.77	0.79	1	
Glu	1.39	0.38		
Gln	0	0.71	1	
Pro	0.84	0	1	
Gly	2.57	2.30	3	1.19
Ala	1.10	0.90	1	0.84
Ile	1.00	1.14	1	0.69
Phe	1.12	1.05	1	
Lys	1.00	0.21	1	
Cofactor	1.00	1.00	1	1.00

^a Values are relative to the amount of cofactor taken, based on its extinction coefficient [2]

Table 2
Automated sequenator analyses of tryptic-chymotryptic peptide

Position	Amino acid	Method of detection		
		By TLC ^a of PTH	By GLC ^a of PTH	TMS-PTH
1	Cys(Flavin)	—	—	—
2	Ile	+	+	+
3	Gly	+	+	n.d.
4	Ala	+	+	n.d.
5	Gly	+	+	n.d.
6	Ser	+	+	+
7	Asp	+	—	+
8	Lys	+	—	+
9	Pro	+	+	+
10	Gly	+	+	n.d.
11	Phe	+	+	+
12	Gln	+	—	+

^a Abbreviations: TLC, thin-layer chromatography; PTH, phenylthiohydantoin; GLC, gas-liquid chromatography; TMS, trimethylsilyl; n.d., not determined

identification. Since the cofactor is phosphorylated [2] it would not be expected to be extracted during the automated sequencing procedure and an appropriate synthetic dansyl derivative is not available for comparison in the manual dansyl-Edman method. Second, the cofactor is linked to a sulfur moiety [15] yet no sulfur amino acid appeared on either acid hydrolysis or was noted in the sequencing studies. Third, in manual sequencing experiments it was noted that no identifiable amino acid appeared at the amino terminus. The absence of a detectable amino acid is characteristic of a residue where flavin is bound [12,13]. The last of these findings is also the reason why cysteinyl flavin is placed at the amino terminus of the above peptides.

Glutamine and phenylalanine were the only amino acids detected on incubation of the tryptic-chymotryptic peptide with carboxypeptidase A. These findings are in agreement with the sequence obtained by the automated sequencing methods.

As expected, after one Edman step on the nagarase peptide, the amino acid composition of the remaining peptide was comparable to that given in table 1, indicating that an unusual amino acid had been removed.

Acknowledgements

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References

- [1] Steenkamp, D. J. and Mallinson, J. (1976) *Biochim. Biophys. Acta* 429, 705–719.
- [2] Steenkamp, D. J. and Singer, T. P. (1976) *Biochem. Biophys. Res. Commun.* 71, 1289–1295.
- [3] Hill, C. L., Steenkamp, D. J., Holm, R. H. and Singer, T. P. (1977) *Proc. Natl. Acad. Sci. USA* 74, 547–551.
- [4] Edmondson, D. E. and Singer, T. P. (1976) *FEBS Lett.* 64, 255–265.
- [5] Colby, J. and Zatman, L. J. (1974) *Biochem. J.* 143, 555–567.
- [6] Steenkamp, D. J., Singer, T. P. and Beinert, H. (1977) *Biochem. J.* in press.
- [7] Matsubara, H. and Sasaki, R. M. (1969) *Biochem. Biophys. Res. Commun.* 35, 175–181.
- [8] Gray, W. (1967) *Meth. Enzymol.* 11, 469–475.

- [9] Ogez, J., Tivol, W. F. and Benisek, W. F. (1977) *J. Biol. Chem.* 252, 6151-6155.
- [10] Pisano, I. J., Bronzert, T. J. and Brewer, H. B. jr (1972) *Anal. Biochem.* 45, 43-59.
- [11] Kulbe, K. D. (1974) *Anal. Biochem.* 59, 564-573.
- [12] Kenney, W. C., Walker, W. H. and Singer, T. P. (1972) *J. Biol. Chem.* 247, 4510-4513.
- [13] Kearney, E. B., Salach, J. I., Walker, W. H., Seng, R. L., Kenney, W., Zeszotek, E. and Singer, T. P. (1971) *Eur. J. Biochem.* 24, 321-327.
- [14] Steenkamp, D. J., Kenney, W. C. and Singer, T. P. (1977) submitted.
- [15] Steenkamp, D. J., McIntire, W. and Kenney, W. C. (1977) submitted.