AMINO ACID SEQUENCE OF L-3-HYDROXYACYL CoA DEHYDROGENASE FROM PIG HEART MUSCLE

Kamal G. BITAR, Agustin PEREZ-ARANDA[+] and Ralph A. BRADSHAW
Department of Biological Chemistry, Washington University School of Medicine, St Louis, MO 63110, USA

Received 9 May 1980

1. Introduction

L-3-Hydroxyacyl CoA dehydrogenase (EC 1.1.1.35) is a mitochondrial enzyme that catalyzes the oxidation of the L(+)-3-hydroxyacyl CoA intermediates formed during the β-oxidation of fatty acids [1]. One mole of NAD^+ is reduced per mole of substrate converted. The enzyme has been partially characterized from sheep [1] and beef liver [2], and a crystalline preparation obtained [3] from pig heart [4]. The method for isolating the protein from the same source was improved and several chemical and physical properties of the enzyme determined [4,5]. As with the distantly related mitochondrial isozyme of malate dehydrogenase [6], it is a basic protein of ~70 000 mol. wt composed of two identical (or highly similar) subunits. Studies to determine the three-dimensional structure by single-crystal X-ray diffraction techniques have been initiated [7].

This communication presents the complete amino-acid sequence of the constituent subunit of the enzyme isolated from pig heart. It contains a total of 307 amino acid residues. A second form, missing the first five amino acids, is present in approximately equal amounts. The origin of the heterogeneity is presently uncertain but probably arises during the process of transferring the enzyme from the cytoplasm, where it is synthesized, to the mitochondrion, where it resides and functions.

2. Materials and methods

2.1. *Preparation of L-3-hydroxyacyl CoA dehydrogenase*

Enzyme was prepared from fresh pig heart by a modification of the method in [4]. Affinity chromatography on 5′-AMP covalently linked to Sepharose 4B (Sigma Chemical Co.) was used as the final step.

2.2. *Enzymatic or chemical hydrolyses*

Peptides for structural analyses were generated from *S*-carboxymethyl enzyme (or fragments derived thereof), prepared as in [8], by trypsin, chymotrypsin, thermolysin (Calbiochem) or *Staphylococcus aureus* V8 protease (Miles Labs). Digestion conditions were maintained in a Radiometer pH stat equipped with a thermostatted reaction vessel. Cleavage with cyanogen bromide (Sigma Chemical Co.) was done in 70% formic acid for 24 h in the dark at room temperature. *o*-Iodosobenzoic acid (Chemalog) was used as in [9].

2.3. *Fractionation of peptides*

Peptides were fractionated by gel filtration or ion-exchange chromatography. Larger fragments, such as those produced by cyanogen bromide or tryptic digestion of citraconylated protein, were separated on columns of Sephadex G-50 or G-100 followed by chromatography on substituted celluloses and Sephadexes. Smaller fragments were purified with substituted polystyrenes using the procedures in [10].

2.4. *Amino acid and sequence analyses*

Amino acid analyses were performed on a Durrum D-500 or Spinco 120C automatic amino acid analyzer following hydrolysis in 6 N HCl at 110°C. All peptides were sequenced automatically in a Beckman 890C Sequencer using 0.1 or 0.33 M Quadrol as coupling buffer. Solvents and reagents were obtained from Beckman Instruments or Burdick and Jackson.

[+] Present address: Department of Biochemistry, University of Madrid, Madrid, Spain

Polybrene (~3 mg) (Abbott) was added, without prior treatment, to each sample prior to analysis. Identification of the phenylthiohydantoins was made by thin-layer chromatography and gas–liquid chromatography. Some residues were assigned by kinetic analyses using carboxypeptidases A, B or Y (Worthington).

3. Results and discussion

The sequence of the L-3-hydroxyacyl CoA dehydrogenase subunit from pig-heart muscle is shown in fig.1. The structure was derived from the automatic sequence analysis of peptides resulting from tryptic, chymotryptic, thermolytic and *S. aureus* protease digests, as well as fragments formed by treatment of the protein with cyanogen bromide and *o*-iodosobenzoic acid. A detailed description of the data used to complete this structure will be given elsewhere.

One of the portions of the structure that was particularly difficult to determine was the amino-terminal region. Sequence analysis of the native (unmodified) protein, which consistently gave better analyses than the *S*-carboxymethylated preparations, showed the presence of two different polypeptides. The identification of the released phenylthiohydantoins for the first 12 cycles is shown in fig.2. Examination of these results reveals that the data are consistent with a mixture of two polypeptide chains differing by the amino-terminal segment:
Ser–Ser–Ser–Ala–Thr–
Quantitation of the released phenylthiohydantoins suggested that the two polypeptides are present in about equal amounts. Subsequent isolation of the corresponding cyanogen bromide or *S. aureus* protease fragments confirmed the assignments shown in fig.2 (lower half). At present it is unclear either how the chains are formed or in what combinations they occur, i.e. homo- and/or hetero-dimers. Inclusion of proteolytic inhibitors in the buffers during purifica-

1 10 20 30 40 50
H_2N-S-S-S-A-T-A-A-A-K-A-K-K-I-L-V-K-H-V-T-V-I-G-G-G-L-M-G-A-G-I-A-Q-V-A-A-A-T-G-H-T-V-V-L-V-D-Q-T-E-D-I-L-

60 70 80 90 100
A-K-S-K-K-G-I-E-E-S-L-R-K-V-A-K-K-K-F-A-E-N-P-K-A-G-D-E-F-V-E-K-T-L-S-S-I-S-T-S-T-D-A-A-A-V-V-H-S-T-D-L-V-

110 120 130 140 150
V-E-A-I-V-E-Q-L-K-V-V-G-E-N-L-K-V-K-S-E-L-F-K-R-L-D-K-F-A-A-E-H-T-I-F-A-S-N-T-S-S-L-Q-I-T-S-L-A-N-A-T-T-R-

160 170 180 190 200 210
Q-D-R-F-A-G-L-H-F-N-V-P-L-M-K-L-V-E-V-V-K-T-P-M-T-S-Q-K-T-L-E-S-L-V-D-F-S-K-T-L-G-K-H-P-V-S-C-K-D-T-P-G-F-

220 230 240 250 260
I-V-N-R-L-L-V-P-Y-L-I-E-A-V-R-L-Y-E-R-G-D-A-S-K-E-D-I-D-T-A-M-K-L-G-A-G-Y-P-M-G-P-F-E-L-L-D-Y-V-G-L-D-T-T-

270 280 290 300 307
K-F-I-I-D-G-W-H-E-M-D-S-Q-N-P-L-F-Q-P-S-P-A-M-N-K-L-V-A-E-N-K-F-G-K-K-T-G-E-G-F-Y-K-Y-K-COOH

Fig.1. The proposed amino-acid sequence for the subunit of pig-heart muscle L-3-hydroxyacyl CoA dehydrogenase. The one-letter code for amino acids is: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.

CYCLE NO.	1	2	3	4	5	6	7	8	9	10	11	12
RESIDUES IDENTIFIED:	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	LEU	ALA	LYS	HIS
	SER	SER	SER	LYS	THR	LYS	LYS	ILE	LYS	VAL	LYS	LYS
PROPOSED ASSIGNMENTS:	SER	SER	SER	ALA	THR	ALA	ALA	ALA	LYS	ALA	LYS	LYS
	ALA	ALA	ALA	LYS	ALA	LYS	LYS	ILE	LEU	VAL	LYS	HIS

Fig.2. Amino-terminal sequence analyses of native L-3-hydroxyacyl CoA dehydrogenase. The upper data set represent the residues identified for each cycle. The lower set is the proposed assignments for the two constituent polypeptides present.

tion did not alter the pattern presented in fig.2, indicating that the heterogeneity is apparently not introduced during isolation. The most likely possibility is that it occurs during the proteolytic processing of a precursor molecule, an event shown to be associated with many, but not all, mitochondrial proteins whose synthesis is nuclear directed [11]. However, the two chains might also be the result of unique genetic information and function, in dimeric combinations, to regulate catalytic activity. A similar heterogeneity of unknown origin was reported for bovine liver rhodanese [12].

By analogy with other NAD^+-dependent dehydrogenases [13], it may be expected that L-3-hydroxyacyl CoA dehydrogenase will contain a cofactor-binding domain and a catalytic domain. These have been tentatively identified by limited homology to occur as the amino- and carboxyl-terminal segments, respectively. This alignment is the same as that found in cytoplasmic malate dehydrogenase and lactate dehydrogenase [13] and is that anticipated for mitochondrial malate dehydrogenase [14]. By similar considerations, His_{200} probably participates in the hydride ion transfer that characterizes the reaction catalyzed. Interestingly, the sole cysteine residue is located at position 204, which may explain the acute sensitivity of this enzyme to thiol reagents [15]. The exact assignment of active site residues as well as the elucidation of evolutionary and mechanistic relationships to other dehydrogenases can be more appropriately accomplished when the three-dimensional structure has been determined. These studies, which should be significantly aided by knowledge of the primary structure, are now in progress.

Acknowledgements

These studies have been supported by a USPHS research grant AM 13362. The authors wish to thank Mr Owen J. Bates of the Washington University Protein Chemistry Facility for valuable assistance in sequence analyses.

References

[1] Lynen, F., Wessely, L., Wieland, O. and Rueff, L. (1952) Angew. Chem. 64, 687–693.
[2] Wakil, S. J., Green, D. E., Mii, S. and Mahler, H. R. (1954) J. Biol. Chem. 207, 631–638.
[3] Stern, J. R. (1957) Biochim. Biophys. Acta 26, 448–449.
[4] Noyes, B. E. and Bradshaw, R. A. (1973) J. Biol. Chem. 248, 3052–3059.
[5] Noyes, B. E. and Bradshaw, R. A. (1973) J. Biol. Chem. 248, 3060–3066.
[6] Noyes, B. E., Glatthaar, B. E., Garavelli, J. S. and Bradshaw, R. A. (1974) Proc. Natl. Acad. Sci. USA 71, 1334–1338.
[7] Weininger, M., Noyes, B. E., Bradshaw, R. A. and Banaszak, L. J. (1974) J. Mol. Biol. 90, 409–413.
[8] Angeletti, R. H., Bradshaw, R. A. and Wade, R. D. (1971) Biochemistry 10, 463–469.
[9] Mahoney, W. C. and Hermodson, M. A. (1979) Biochemistry 18, 3810–3814.
[10] Bradshaw, R. A., Bates, O. J. and Benson, J. R. (1980) J. Chromatog. 187, 27–33.
[11] Schatz, G. (1979) FEBS Lett. 103, 203–211.
[12] Weng, L., Russell, J. and Heinrikson, R. L. (1978) J. Biol. Chem. 253, 8093–8101.
[13] Rossmann, M. G., Liljas, A., Bränden, C.-I. and Banaszak, L. J. (1975) The Enzymes 3rd edn, 11, 61–102.
[14] Banaszak, L. J. and Bradshaw, R. A. (1975) 3rd edn, 11, 369–396.
[15] Bradshaw, R. A. and Noyes, B. E. (1975) Methods Enzymol. 35, 122–128.