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Purification and Characterization of Bovine Liver 3-*cis*-2-*trans*-Enoyl-CoA Isomerase

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Summary: The purification to homogeneity of 3-*cis*-2-*trans*-enoyl-CoA isomerase from bovine liver is described. This procedure has also been successfully applied to the isolation of the enzyme from rat liver mitochondria. Its molecular mass was determined to be 30 kDa by several methods. K_m of the isomerase for 3-*cis*-dodecenoyl-CoA, the physiological intermediate in the β-oxidation of oleic acid and of the 3-*trans*-isomer was determined to be 3.2×10^{-5}M. The velocity of isomerization of the 3-*trans*-substrate is reduced 10–15 times. Inhibition experiments and pH-dependency of the reaction kinetics suggest the participation of a histidine residue in the isomerization. Protein analytical studies revealed that the N-terminus is blocked (acetylated). Cyanogen bromide cleavage of the purified enzyme resulted in five fragments. The N-terminus of one fragment is blocked. Partial amino-terminal sequences of two of the fragments were obtained by Edman degradation, a prerequisite for further studies on the structure of this isomerase on the DNA level.

*Reinigung und Charakterisierung der Rinderleber-3-*cis*-2-*trans*-Enoyl-CoA-Isomerase*

Zusammenfassung: Die Reindarstellung der 3-*cis*-2-*trans*-Enoyl-CoA-Isomerase aus Rinderleber bis zur Homogenität wird beschrieben. Nach der gleichen Vorschrift gelingt ebenso die Isolierung des Enzyms aus Rattenlebermitochondrien. Die Molekularmasse wurde nach mehreren Methoden zu 30 kDa bestimmt, K_m für 3-*cis*-Dodecenoyl-CoA beträgt 3.2×10^{-5}M. Die Isomerisierungsgeschwindigkeit des 3-*trans*-Substrats ist 10–15fach langsamer. Inhibierungsversuche und pH-Abhängigkeit der Reaktionsgeschwindigkeit weisen auf die Beteiligung eines Histidinrestes an der Isomerisierung hin. Proteinchemische Analysen zeigten, daß der N-Terminus blockiert (acetyliert) ist. Die Bromcyan-Spaltung ergab fünf Fragmente, von denen eines nicht sequenzierbar war. Aminoterminale Teilsequenzen von zwei Fragmenten durch Edman-Abbau dienen weiteren Studien zur Struktur dieser Isomerase auf der DNA-Ebene.

Key words: Mitochondrial β-oxidation, unsaturated fatty acids, isomerization, enzyme kinetics, protein analysis.

Naturally occurring mono- and polyunsaturated fatty acids comprise about half of the acyl components of triglycerides, phospholipids and cholesterol esters. Their double bonds are methylene interrupted, have all-*cis* configuration either on odd or even numbered carbon atoms in polyunsaturated fatty acids of the

Enzymes:
3-*cis*-2-*trans*-Enoyl-CoA isomerase, recommended name: dodecenoyl-CoA Δ^3-*cis*-Δ^2-*trans*-isomerase (EC 5.3.3.8); 3-Hydroxyacyl-CoA dehydrogenase, (*S*)-3-hydroxyacyl-CoA:NAD® oxidoreductase (EC 1.1.1.35); Enoyl-CoA hydratase, (3*S*)-hydroxyacyl-CoA hydro-lyase (EC 4.2.1.17); D(–)-3-hydroxyacyl-CoA epimerase (EC 5.1.2.3).

Abbreviations:
PAGE, polyacrylamide gel electrophoresis; PTH, phenylthiohydantoine; SDS, sodium dodecyl sulfate.

palmitoleic, oleic, linoleic and α-linolenic acid families. The β-oxidation of these double-bond systems requires additional mitochondrial enzymes. The *cis*-double bonds on odd C-atoms yield 3-*cis*-enoyl-CoA ester as intermediates. During β-oxidation they are geometrically and positionally isomerized by the 3-*cis*-2-*trans*-enoyl-CoA isomerase to the 2-*trans*-enoyl-CoA intermediates and then hydrated by the enoyl-CoA hydratase to L(+)-3-hydroxyacyl-CoA[1,2]. We described the purification to homogeneity of rat 3-*cis*-2-*trans*-enoyl-CoA isomerase. The enzyme has an estimated molecular mass of 30 kDa[3].

In order to obtain more information about the enzymatic properties and the protein structure of this key enzyme in the β-oxidation of unsaturated fatty acids we used bovine liver as an enzyme source for the purification on a preparative scale. In this communication we describe the purification of the enzyme to homogeneity in polyacrylamide gel electrophoresis and some of its enzymatic properties including studies on the catalytic site. Reaction mechanisms of the isomerization of the double bond are discussed.

Cyanogen bromide fragmentation of the 30-kDa protein yielded five fragments, two of which were submitted to Edman degradation. The N-terminus of the enzyme is blocked.

The purification steps for the bovine liver enzyme has also been applied to its isolation from rat liver mitochondria. The specific activity in the crude extract was twice that of the bovine enzyme, 33 and 15 U/g, respectively; the rat isomerase, however, is very labile in the acid dialysis step. The enzyme from both sources showed no significant difference in molecular mass of 30 kDa under denaturing conditions of the SDS polyacrylamide gel electrophoresis.

The enzyme elutes as dimer with a molecular mass of 60 ± 3.0 kDa in gel filtration on Sephadex G-150 with a Stoke's radius determined by gel filtration.

Table 1. Procedure for the purification of 3-*cis*-2-*trans*-enoyl-CoA isomerase from bovine liver.

I	Homogenization of minced fresh bovine liver (600 × *g*) in 25mM Na-phosphate buffer pH 7.4, 1mM EDTA (buffer A, 2400 m*l*), with Waring blendor 0.5 min at low, 2.5 min at highest speed ↓ Centrifugation at 600 × *g*, 10 min Supernatant ↓ Centrifugation at 20000 × *g*, 20 min Supernatant (1800 m*l*) ↓ $(NH_4)_2SO_4$ saturation to 60%, centrifugation at 20000 × *g*, 20 min Sediment ↓ Resuspension in buffer A (800 m*l*), heat step (69 °C in boiling water, rapid cooling in ice bath) in aliquots of 175 m*l*, centrifugation at 20000 × *g*, 20 min
II	Crude extract (supernatant, 550 m*l*) ↓ Dialysis against 25mM Na-phosphate buffer pH 7.4 change to 25mM Na-phosphate buffer pH 6.0, centrifugation at 20000 × *g*, 20 min
III	CM Cellulose (CM52) chromatography I (gel bed 500 m*l*) of supernatant, adsorption rate 100 m*l*/h, unbound enzyme ↓ Dialysis against 15mM Na-phosphate buffer pH 6.0, 1mM EDTA (buffer D), adsorption to CM cellulose
IV	CM Cellulose chromatography II (gel bed 2150 m*l*), adsorption rate 50–70 m*l*/h ↓ Linear gradient elution with buffer D to 0.3M NaCl in buffer D, 10-m*l* fractions, dialysis of peak fraction, Amicon ultrafiltration and concentration to 2–6 m*l*
V	Sephadex G-150 chromatography (2 × 140 cm), 4-m*l* fractions ↓ Dialysis of peak fractions against buffer E
VI	ω-Aminohexylagarose chromatography (1.8 × 25 cm) ↓ Linear gradient (60 m*l*): buffer E to 0.3M NaCl in buffer E Purified enzyme

Experimental

Purification of the enzyme

The flow sheet (Table 1) summarises the purification steps leading to the purified isomerase. Beef liver from the local slaughter house or liver from adult, healthy rats was used as enzyme source. All steps were carried out at 4 °C. All buffers used contained 0.2 g NaN_3/*l*.

Homogenisation, $(NH_4)_2SO_4$ precipitation, heat step

The tissue was homogenised in a hypotonic phosphate buffer (25mM sodium phosphate, pH 7.4, 1mM EDTA) in a Waring blendor for 0.5 min at low and 2.5 min at highest speed.

Nuclei and cell debris were removed by centrifugation (600 × *g*, 10 min and 20000 × *g*, 20 min). The enzyme was precipitated at 60% $(NH_4)_2SO_4$ saturation. The precipitate was pelleted by centrifugation at 20000 × *g* for 20 min and dissolved in homogenisation buffer. The solution was heated in a boiling water bath with thorough stirring and rapidly cooled in ice when the temperature had reached 69 °C. Heat denatured proteins were removed by centrifugation and the supernatant dialysed against 25mM Na-phosphate, pH 7.4 and then 25mM Na-phosphate, pH 6.0.

Cellulose CM 52 chromatography I and II

The dialysed protein solution was applied at a protein to gel ratio of 1 g/40–70 m*l* cellulose. Under these conditions all cytochromes representing 10–20% of the contaminating protein, were effectively removed. The enzyme solution was applied to a second cellulose CM52 column at a protein to gel ratio of 1 g/140–190 m*l* cellulose and eluted by a linear gradient consisting of 2 × 0.5 *l* of 15mM Na-phosphate, pH 6.0, 1mM EDTA, and 0.3M NaCl in the same buffer. The fractions of highest specific activity were pooled and dialysed against 25mM Na-phosphate, pH 7.4.

Sephadex G-150, ω-aminohexylagarose chromatography

The enzyme fractions were concentrated in ultra thimbles (Schleicher & Schüll, Düren) to a volume of 2–6 m*l*. The solution

was applied to a Sephadex G-150 column (2.4 × 135 cm). Enzyme-containing fractions were pooled and finally purified on a ω-aminohexylagarose column equilibrated with 50mM Na-phosphate, pH 7.4, 3mM EDTA, 10mM mercaptoethanol (buffer E). The enzyme was eluted by a linear gradient from 0 to 0.3M NaCl in the same buffer.

Enzyme assay

3-*cis*-Dodecenoyl-CoA was prepared by the mixed anhydride method[1,4,5] and the concentration determined by the method of Elman[6]. The isomerisation of the double bond of the substrate was measured at 263 nm ($\varepsilon = 6.2 \times 10^3 M^{-1} \times cm^{-1}$[13]).

Inhibition of the enzyme by diethylpyrocarbonate

Diethylpyrocarbonate (Sigma) was stored over $CaCl_2$ in the cold and diluted ten-fold with absolute ethanol and just before being used ten-fold with buffer (50mM Na-phosphate, pH 7.0). The final ethanol concentration in the assay solution was below 0.1%. The enzyme was incubated with diethylpyrocarbonate at room temperature.

Cyanogen bromide cleavage of 3-cis-2-trans-enoyl-CoA isomerase

1 mg of the purified enzyme dissolved in 70% formic acid was cleaved with 20 mg CNBr for 15 h at room temperature in the dark. The fragments were separated by PAGE (17.5%)[7] and also by HPLC on Lichrosorb Si 60 and Si 100 with 90% formic acid as solvent[8–10]. Automated Edman degradation of the fragments[11] and HPLC of the PTH amino acids were carried out as described earlier[12].

Results

Purification of 3-cis-2-trans-enoyl-CoA isomerase

The enzyme was isolated from bovine liver and obtained in purified form in a six step purification procedure. These steps, yields and enrichment factors are presented in Table 2, the PAGE analysis of the fractions of the ω-aminohexylagarose chromatography in Fig. 1.

Kinetic studies

3-*cis*- and 3-*trans*-Dodecenoyl-CoA esters synthesised by the mixed anhydride method[1,4,5] were used

Table 2. Purification steps leading to homogeneous 3-*cis*-2-*trans*-enoyl-CoA isomerase.

Purification step	Activity [U]	Protein [mg]	Spec. activ. [U/mg]	Recovery [%]	Purification factor[1]
Crude extract[2]	8984	7456	1.2	100	1
CM cellulose I	7728	5044	1.5	86	1.3
CM cellulose II	1878	230	8.2	21	6.8
Sephadex G-150	254	91	28.2	2.8	23.5
ω-Aminohexylagarose	197	2.1	93.8	2.2	78

[1] Purification factors are related to the specific activity of the crude extract (see flow sheet under Experimental).
[2] Crude extract results from the $(NH_4)_2SO_4$ precipitation, heat step and acid dialysis of bovine liver homogenate.

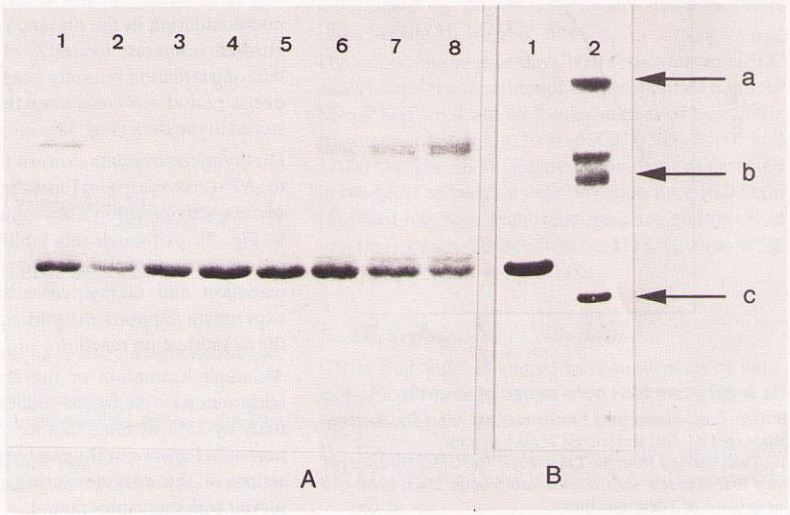

Fig. 1A: SDS-Polyacrylamide gel electrophoresis (12.5%) of the crude extract (see Table 1) (lane 1) and of fractions of the final ω-aminohexylagarose chromatography (lanes 2–8).

B: 1, Purified isomerase (I); 2, marker proteins: a, BSA (66 kDa); b, ovalbumin (45 kDa); c, apolipoprotein AI (28 kDa).

as substrates. The isomerization of the 3-*cis*- or 3-*trans*-enoyl-CoA to the 2-trans-enoyl-CoA derivative was monitored by the appearance of the 263 nm absorption maximum of the latter. From the Lineweaver-Burk diagram the Michaelis-Menten constant for 3-*cis*-dodecenoyl-CoA was determined to be $K_m = 3.2 \times 10^{-5}$M and $V_{max} = 244\ \mu mol \times min^{-1} \times mg^{-1}$, which corresponds to a turnover number of 7.7×10^3 min^{-1} assuming a molecular mass of 30 kDa.

The K_m of the rat liver isomerase has been determined to be 5×10^{-5}M[13] and that of the bovine enzyme in a crude extract 1×10^{-5}M[14].

The substrate affinity is therefore of the same order of magnitude as for other β-oxidation enzymes, e.g. enoyl-CoA-hydratase[15], β-hydroxyacyl-CoA-dehydrogenase[16] and D(−)-3-hydroxyacyl-CoA epimerase[17]. A K_m of the 3-*trans*-dodecenoyl-CoA of 2.1×10^{-5}M and $V_{max} = 19\ \mu mol \times min^{-1} \times mg^{-1}$ was deduced from the Lineweaver-Burk plot.

Enzyme inhibition experiments

Free 2-*trans*-dodecenoic acid and the 3-dodecynoic acid inhibit the isomerization of the naturally occurring 3-*cis*-dodecenoyl-CoA ester competitively as revealed by the Lineweaver-Burk double reciprocal plot with five different substrate and three different inhibitor concentrations. Their inhibition constants were deduced from the Eadie-Hofstee plot[18]: $K_i = 5.2 \times 10^{-5}$M for 3-*trans*-dodecenoic acid and 3.5×10^{-5}M for 3-dodecynoic acid. Coenzyme A also competitively inhibits, $K_i = 3.3 \times 10^{-5}$M. The very similar inhibition constants of the free fatty acid and free coenzyme A refer to the apparently equal structural prerequisite for optimal enzymatic catalysis. This is supported by our earlier finding that *N*-acetylcysteamine derivatives of 3-*cis*-unsaturated fatty acid lack essential structural prerequisites for an optimal reaction.

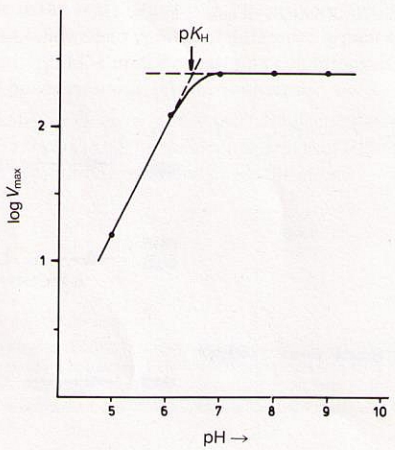

Fig. 2. pH Dependence of the maximal reaction rate (V_{max}).
Buffers used were: 25mM Na-acetate (pH 4.0, 5.0), Na-phosphate (pH 6.1, 7.0) and Tris/HCl (pH 8.0, 9.0).
V_{max} was derived from the Lineweaver-Burk plot for each pH with four different substrate concentrations. Each point is a mean value of 3 determinations.

pH Dependence of the isomerase reaction

The two proposed mechanisms require the participation of a basic group which is reversibly protonated and deprotonated. Therefore the pH dependence of the reaction rate was measured between pH 5 and 9 to obtain information about the participating side chain (Fig. 2). A $K_H = 3.2 \times 10^{-7}$M corresponding to a $pK_H = 6.5$ was deduced. This value strongly favours to a histidine residue as an acceptor of a proton from the C-atom adjacent to the carbonyl group (mechanism B in Fig. 5) with an intramolecular proton transfer from C-2 to C-4.

Chemical modification reaction of the isomerase

Further support for the participation of a histidine group at the catalytic site came from two chemical modification reactions with the 3-*cis*-enoyl-CoA isomerase: a) the photooxidation of the histidine residue at neutral pH in the presence of Rose Bengal[19,20] and b) reaction with diethylpyrocarbonate[21]. Fig. 3a illustrates the loss of enzymatic activity upon photooxidation in the presence of Rose Bengal. The purified isomerase lost 60% of its activity within 5 min of irradiation but only less than 30% in the dark over a period of 70 min when the control sample was stored in the dark (Fig. 3a).

Diethylpyrocarbonate, known to derivatise histidine to N^{im}-(ethoxycarbonyl)histidine, reduced the isomerase activity within a few minutes as demonstrated in Fig. 3b. Although this inhibitor also reacts with tyrosine, tryptophan and lysine residues, the photooxidation and diethylpyrocarbonate derivatisation experiment supports the participation of histidine in the isomerization reaction.

Although incubation of the enzyme with 10^{-5}M 4-(chloromercuri)benzoate reduces the enzymatic activity by 75% within 2 min we exclude the participation of SH groups in the catalytic site since no stabilisation of the enzyme during purification was observed with mercaptoethanol.

Comparison of pig and bovine liver isomerase

An acetylene-allene isomerase from pig liver has been described and postulated to be identical with the 3-*cis*-2-*trans*-enoyl-CoA isomerase[22,23]. We investigated whether pig liver only contains the acetylene-allene isomerase or in addition also the 3-*cis*-2-*trans*-enoyl-CoA isomerase activity. The two enzymes were isolated from pig liver according to Miesowicz and Bloch (method A)[23] and as described in this communication (method B). 3-Dodecynoyl-*N*-acetyl-cysteamine[24] was used as substrate for the acetylene-allene isomerase[22,23].

Table 3 compares the enzyme activities of bovine and pig liver acetylene-allene isomerase and 3-*cis*-2-*trans*-enoyl-CoA isomerase. These results disprove the suggestion[23] that both isomerases might be identical.

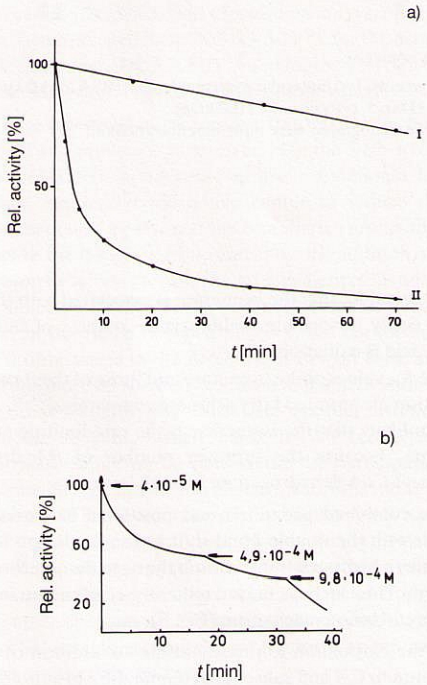

Fig. 3. a) Photooxidation of 3-*cis*-2-*trans*-enoyl-CoA isomerase.
I, Control (radiated without Rose Bengale)
II, Photo-oxidation of the isomerase in the presence of 16.5 μg Rose Bengale/ml.
Irradiation was done with white light.
b) Inhibition of the enzyme by diethylpyrocarbonate at the indicated concentrations.

Table 3. Comparison of the activities of 3-*cis*-2-*trans*-enoyl-CoA isomerase (substrate: 3-*cis*-dodecenoyl-CoA) and acetylene-allene isomerase (substrate: 3-dodecynoyl-CoA) in different enzyme preparations.

Species	Method	Enoyl CoA isomerase [U/g]	Acetylene-allene isomerase [U/g]
Pig	A	1.4	30.7
Pig	B	1.7	7.6
Cattle	A	9.4	1.8
Cattle	B	15.5	–

*Characterization of the bovine liver 3-*cis*-2-*trans*-enoyl-CoA isomerase*

Molecular mass determination by gel exclusion chromatography on Sephadex G-150 indicates a molecular mass of 60000 ± 3000 Da, in Ultragel AcA44 two third eluted as the monomeric form with 30000 Da, the rest as dimer, both forms with the same specific activity.

The molecular mass of 30000 ± 1500 Da was confirmed by SDS polyacrylamide gel electrophoresis (12.5% acrylamide). Attempts to determine N-terminal end groups of the isomerase by the method of Hartley[25] or by the Edman degradation failed. Also treatment of the isomerase with pyroglutamyl aminopeptidase (5-oxoprolyl-peptidase, EC 3.4.19.3)[26] released no free N-terminus.

Cyanogen bromide cleavage of the isomerase

Treatment of the enzyme with cyanogen bromide released five fragments. Their molecular masses were determined by polyacrylamide gel electrophoresis (Fig. 4a) and HPLC (Fig. 4b).

The retention times of the CNBr fragments in HPLC analysis and the R_F values of the polypeptide bands in SDS-PAGE indicate molecular masses of five CNBr fragments of roughly 3000, 5000, 6500, 8500, and 11000 Da. The 3-kDa fragment could not be detected in the gel. The fraction with retention time 10.07 min (Fig. 4b) has been rechromatographed and resolved into three bands (10.27 min ≡ 6.5 kDa, 11.0 min ≡ 5.0 kDa and 9.85 min ≡ 8.0 kDa).

Edman degradation of CNBr fragments

CNBr fragments II and VI were sequenced by automated Edman degradation over 11 and 12 cycles, respectively. The sequence of the fragments is:

II: Lys-Leu-Leu-Asn-Leu-Pro-Ile-Tyr-Leu-Ala-Ser-
VI: Ala-Arg-Trp-Leu-Ala-Val-Pro-Asp-His-Ala-Arg--Gln-

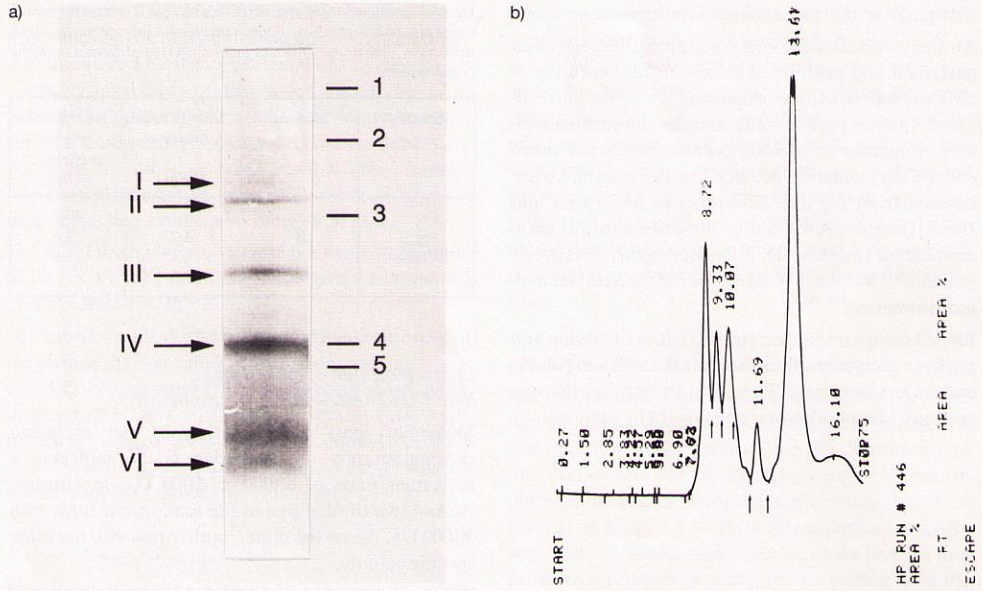

Fig. 4. Cyanogen bromide cleavage of purified 3-*cis*-2-*trans*-enoyl-CoA isomerase.

a) Polyacrylamide gel electrophoresis, 17.5% slab gel[7]. I–IV CNBr fragments. 1–5 molecular mass standards: 1, BSA (66 kDa); 2, ovalbumin (45 kDa); 3, apolipoprotein AI (28 kDa); 4, lysozyme (14.3 kDa); 5, cytochrome *c* (11.7 kDa).

b) HPLC separation of CNBr fragments of isomerase. Conditions for chromatography were those described before[8–10].

Discussion

The isolation of the 3-*cis*-2-*trans*-enoyl-CoA isomerase from liver had to overcome major obstacles:

1) its low expression in the bovine liver. We estimated by in vitro translation of total bovine liver mRNA combined with immunoprecipitation, that no more than 0.05–0.1% of the RNA are isomerase-specific RNA transcripts.
2) The isolation of bovine liver mitochondria by the regular cell fractionation procedures is less satisfactory because of the fibrous interstitial tissue.
3) The purification steps of the active enzyme proceedes with losses of enzyme protein and enzyme activity to some extent. Particularly the last step, the efficient ω-aminohexylagarose chromatography, leads to a high dilution and due to the hydrophobicity of the enzyme protein to losses by adsorption to surfaces. The hydrophobic properties are also documented by the dimerization, activation by Triton X-100 and its binding to the inner mitochondrial membrane, which we observed during mitochondrial fractionation studies. It is conceivable, that the isomerase is associated with the easily dissociable multienzyme complex of fatty acid β-oxidation.

The K_m values of the isomerase and those of the β-oxidation of saturated fatty acids are comparable[27,28]. It is unlikely that the isomerase is the rate limiting enzyme, because the turnover number of β-hydroxyacyl-CoA dehydrogenase is much lower.

The combined geometric and positional isomerization with the double bond shift to the 2-position requires a hydrogen transfer from the α- to the γ-carbon atom. This can be achieved either by a carbonium ion or a carbanion mechanism (Fig. 5).

In the carbonium ion mechanisms an addition of a proton to C-4 and subsequent elimination of a proton at C-1 prohibits an intramolecular proton transfer. A similar mechanism has been proposed for the isopentenyldiphosphate Δ^3–Δ^2-isomerase (EC 5.3.3.2)[29]. In the carbanion mechanism a proton is abstracted from C-2 by a basic group in the active center of the enzyme with the subsequent reprotonation of C-4 without proton exchange with water. The term "base-

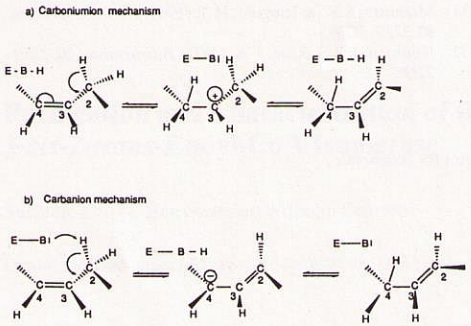

Fig. 5. Proposed reaction mechanism of the 3-*cis*-2-*trans*-isomerization.

catalysed allylic proton transfer" has been given to this mechanism and was demonstrated with the structurally related reaction of the vinylacetyl-CoA isomerase (EC 5.3.3.3) in *Clostridium kluyveri*[30], for the 5-steroid isomerase (EC 5.3.3.1)[31], for the aconitate isomerase (EC 5.3.3.7)[32] and for the acetylen-allene isomerase[23] by the kinetic isotope effect.

A base-catalysed allylic proton transfer in the positional and geometric isomerization of the 3-*cis*- to the 2-*trans*-double bond could utilize a functional histidine residue in the active center to induce the abstraction of a proton at the α-methylen group adjacent to the thioester group and allow the addition of a proton to carbon atom 4. The involvement of histidine is indicated by the pH-dependence of the isomerization in the range between pH 6–8, the photooxidation of histidine in the presence of Bengal Rose and furthermore by the derivatisation and inactivation of histidine by diethylpyrocarbonate, another histidine-specific reagent, which leads to N^{im}-(ethoxycarbonyl)histidine. We do not assume the participation of free SH-groups in the isomerization reaction, although 10^{-5}M 4-chloromercuribenzoate leads to a 50% inhibition within 1 min. However enzyme activity remains unimpaired in the absence of thiols over the multistep purification. Also the pK of the SH group lies beyond pH 8. Therefore we assume that intact SH-groups are required for the correct conformation or the substrate binding.

The mature bovine and rat liver 3-*cis*-2-*trans*-enoyl-CoA isomerase has an apparent molecular mass of 30 kDa. In vitro transcription studies indicate that the enzyme is nuclear encoded and the primary translation product is 32 kDa large, thus equipped with a signal peptide of 20–30 amino-acid residues essential for the mitochondrial import (unpublished results).

The chemical degradation of the purified isomerase by cyanogen bromide and the isolation of tryptic cleavage products and the available peptide sequences of these fragments not only indicate a rather large homology between the bovine and rat isomerase but will make available the complete primary structure of the bovine isomerase, particularly in view of the rat liver 3-*cis*-enoyl-CoA isomerase-specific cDNA sequence which has been determined in this laboratory (Müller-Newen, G. & Stoffel, W., in preparation).

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