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ACETYL-CoA ACETYLTRANSFERASE FROM BOVINE LIVER MITOCHONDRIA

MOLECULAR PROPERTIES OF MULTIPLE FORMS

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Summary

Bovine liver mitochondrial acetyl-CoA acetyltransferase (acetyl-CoA:acetyl-CoA *C*-acetyltransferase, EC 2.3.1.9) has been obtained in three forms designated transferase I, A and B on the basis of their elution positions from chromatography on phosphocellulose. All forms have been shown to have a molecular weight of about 152 000, each being composed of four similar subunits. Amino acid analysis of transferase A and B, the two major forms, revealed a close relationship between both forms with almost identical amino acid composition and arginine as N-terminal residue.

The three transferases differ with respect to their redox state and their multiplicity of forms with isoelectric points of 6.9, 7.5 and 8.8, into which the transferases I and A were spontaneously transformed upon isoelectric focusing or rechromatography on phosphocellulose. Transferase B represents a stable enzyme form with an isoelectric point of 8.8. Although the redox state of transferase B can be adjusted to that of transferase A still a difference in charge and in the multiplicity of forms exists, thus indicating different protein states.

Introduction

Ketogenesis occurs exclusively in mitochondria [1,2]. The formation of ketone bodies from various substrates does not correlate with the mitochondrial concentration of acetyl-CoA, but rather with the acetyl-CoA/CoA-SH ratio [3—5]. The mitochondrial acetyl-CoA acetyltransferase (acetyl-CoA:acetyl-CoA *C*-acetyltransferase, EC 2.3.1.9) catalyzes the synthesis of acetoacetyl-CoA, the first step in the biosynthesis of ketone bodies [6]. In liver mitochondria, as first demonstrated by Middleton [7,8], this enzyme has been found to occur in two chromatographically distinct forms, enzyme A and enzyme B.

Kinetic analysis and studies on the regulatory properties of these two forms have clearly shown that acetyl-CoA acetyltransferase catalyzes a regulatory step in the biosynthesis of ketone bodies [9,10].

The present study which is concerned with the number and molecular properties of the multiple forms of the mitochondrial acetyl-CoA acetyltransferase, describes three different forms.

Materials and Methods

Enzymes and substrates. Enzymes and biochemicals were purchased from Boehringer Mannheim GmbH (Mannheim, Germany) or from Merck (Darmstadt, Germany). 2,3-*trans*-Hexenoic acid was from Roth (Karlsruhe, Germany); dithioerythritol and 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) from Sigma (München, Germany); chemicals for polyacrylamide gel electrophoresis, Servalyt and dansyl amino acids from Serva (Heidelberg, Germany); Phosphocellulose and DEAE-cellulose from Schleicher and Schüll (Dassel, Germany); QAE-Sephadex, Sephadex A-25, G-10 and G-200 from Pharmacia (Frankfurt, Germany). Acetyl-CoA, acetoacetyl-CoA, 2,3-*trans*-Hexenyl-CoA were prepared, purified and assayed as described earlier [10].

Enzymes assays. The activity of acetyl-CoA acetyltransferase in the direction of acetoacetyl-CoA cleavage was followed in an assay system (1 ml) containing 100 mM Tris (pH 8.1), 5 mM $MgSO_4$, 0.05 mM acetoacetyl-CoA and 0.6 mM CoA at 30°C and 313 nm using $\epsilon = 9570\ M^{-1} \cdot cm^{-1}$ *.

With 3-oxohexanoyl-CoA as substrate, a pH of 8.1 and 5 mM $MgSO_4$ were employed according to a modified method of Seubert et al. [11] using $\epsilon = 9050\ M^{-1} \cdot cm^{-1}$.

Enzyme purification. The mitochondrial acetyl-CoA acetyltransferase was isolated from bovine liver according to Huth et al. [10], but supplemented by chromatography on DEAE-cellulose and gel filtration on Sephadex G-200 and using sodium phosphate buffer with 10 mM mercaptoethanol or 5 mM dithioerythritol (step 6), 1 mM EDTA and 20% (w/v) glycerine, throughout the purification procedure instead of potassium phosphate buffer, since potassium acts as an activator of the mitochondrial acetyl-CoA acetyltransferase [8]. Extraction of mitochondria (step 1), fractionation with $(NH_4)_2SO_4$ (step 2), chromatography (step 4) and rechromatography on phosphocellulose (step 6) were performed as described earlier [10].

The additional steps were carried out as follows: DEAE-cellulose chromatography (step 3): The $(NH_4)_2SO_4$ fraction was dialyzed against 0.01 M sodium phosphate buffer (pH 7.7) and applied to a DEAE-cellulose column (10 mg protein per ml packed volume) equilibrated with 0.01 M sodium phosphate (pH 7.7) and eluted with the same buffer (10-ml fractions). Protein concentrations were monitored at 280 nm and enzyme activities estimated, with acetoacetyl-CoA and 3-oxohexanoyl-CoA, in each fraction. Thus the elution of both enzymes the acetyl-CoA acetyltransferase and acetyl-CoA acyltransferase through the DEAE-cellulose column could be followed. Sephadex G-200 chromatography (step 5): The enzyme protein sample was applied to a column

* This coefficient given in ref. 10 was incorrect.

(2.5 X 100 cm), equilibrated with 0.05 M sodium phosphate (pH 7.2) and eluted at a flow rate of 3.5 ml/h. The protein was determined by the method of Lowry et al. [12], standardized by dry weight determinations of a sample of dialyzed acetyl-CoA acetyltransferase.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis was performed in columns (0.6 X 6 cm) of 7.5% acrylamide using a continuous buffering system of 50 mM Tris/glycine (pH 8.9), 15 mM 2-mercaptoethanol (cathode) and 100 mM Tris/glycine (pH 8.1) (anode) for 4 h at 2 mA/column. Gels were stained for 10 min at 60° C in a staining solution (0.115 g Coomassie Brillant Blue R 250 in 100 ml destaining solution) and destained by diffusions against 8% acetic acid in 25% ethanol.

Molecular weight. A Sephadex G-200 column (1.5 cm X 90 cm) equilibrated with 0.05 M sodium phosphate (pH 7.2), 5 mM dithioerythritol. 20% (w/v) glycerine was calibrated with the following reference proteins (molecular weight in parentheses): Aldolase (155 000), catalase (240 000), bovine serum albumin (68 000), ovalbumin (45 000), chymotrypsinogen A (25 000) and cytochrome *c* (12 500); flow rate, 1 ml/h. 1 mg purified enzyme was applied in 1 ml to the column along with 2 mg cytochrome *c* (as internal marker) and eluted under the same conditions as the calibrating mixture [13]. Molecular weight calculations were performed by the least-squares method.

Sedimentation equilibrium analysis of the purified enzymes were performed in a Spinco Model E analytical ultracentrifuge from Beckman.

Molecular weights of the subunits were determined by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis [14].

Amino acid composition, identification of NH_2-terminal residue and determination of sulfhydryl groups. Approx. 0.5-mg samples were hydrolyzed in 6 M HCl at 110°C in a sealed evacuated tube for 24, 48 and 72 h. The amino acid analysis was carried out on a Beckman Unichrom Amino Acid Analyzer by the method of Moore and Stein [15]. Tryptophan content was estimated spectrophotometrically by the method of Gaitonde and Dovey [16]. Half-cystine content was determined as cysteic acid according to Moore [17]. The NH_2-terminal residues were identified by the method of dansylation [18].

The number of sulfhydryl groups per mol enzyme was determined after treatment with 5,5'-dithiobis-(2-nitrobenzoic acid) photometrically at 410 nm [19].

Isoelectric focusing. Isoelectric points (p*I*) were measured at 4°C by isoelectric focusing in an LKB Model 8101 electrofocusing column (capacity 110 ml). A 20—60% (v/v) glycerine density gradient, containing 1% ampholine carrier ampholyte of a pH range from 6 to 10 was formed above the cathode. The enzyme protein was adjusted in a small volume to the proper density (40%, v/v) and added directly to the column when half of the gradient had been formed.

Results

When mitochondrial acetyl-CoA acetyltransferase was subjected to phosphocellulose chromatography, the enzyme activity was resolved into one minor peak and two major peaks, designated transferases I, A and B (Fig. 1). These activity peaks were eluted at 0.35 M (Na^+), 0.42 M (Na^+) and 0.49 M (Na^+) for

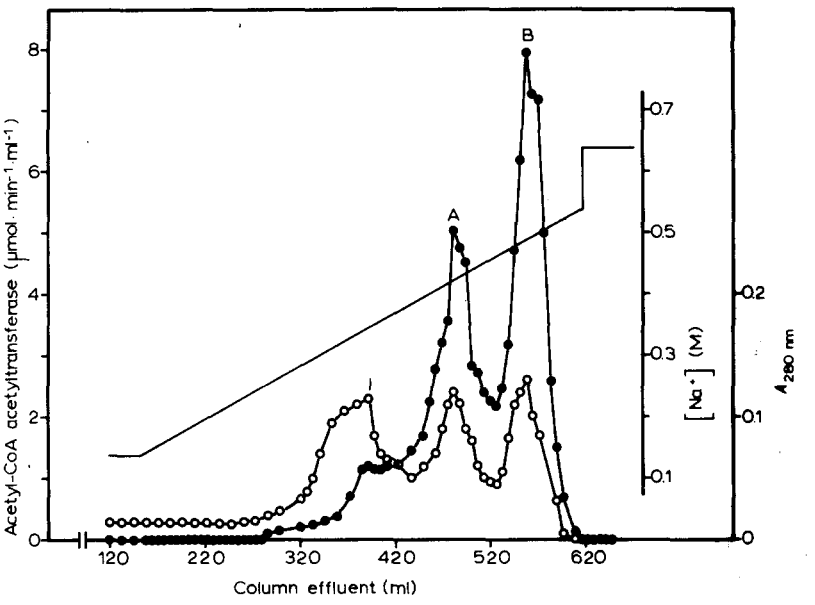

Fig. 1. Resolution of the mitochondrial acetyl-CoA acetyltransferase by rechromatography on phosphocellulose (step 6 of enzyme purification). 138 mg protein were applied in 1 ml buffer to a 1.5 × 30 cm phosphocellulose column, previously equilibrated with 0.1 M sodium phosphate buffer (pH 6.6) employing a linear gradient of 290 ml 0.1 M sodium phosphate buffer (pH 6.6) (Na^+ = 0.137 M) and 0.4 M NaCl in 0.1 M sodium phosphate buffer (pH 6.6) (Na^+ = 0.537 M). Specific enzyme activities were 15, 58 and 66 units per mg protein for the transferases I, A and B, respectively. ○, absorbance at 280 nm; ●, enzyme (units · ml^{-1}) towards acetoacetyl-CoA; ———, Na^+ concentration (M) as measured by conductivity.

transferases I, A and B and contained 12.5, 37.5 and 50% of the total enzyme activity, respectively. Purification in presence of phenylmethanesulfonyl fluoride, an inhibitor of serine-dependent proteases did not affect the elution pattern of the phosphocellulose chromatography.

Polyacrylamide gel electrophoresis pattern

In presence of 15 mM 2-mercaptoethanol, transferases I and A showed two protein bands in polyacrylamide gel electrophoresis, whereas transferase B exhibited only one protein band (Fig. 2). Each protein band coincides with enzymatic activity. Electrophoretic mobilities of the different forms are closely related to their elution positions on phosphocellulose: form B migrated faster to the anode than the two other forms. Based on polyacrylamide gel electrophoresis in the presence and absence of SDS, each form appears to be homogeneous (Fig. 3).

Free sulfhydryls, amino acids composition and NH_2-terminal residues

In presence of SDS, the transferases I, A and B were shown to contain 10.9, 20.5 and 14.3 mol sulfhydryl per mol enzyme, respectively (Table I). Concerning the accessibility of sulfhydryl groups, transferase A and B do not differ from each other. However, the difference in the content of total free sulfhydryl groups between the two main forms A and B was statistically significant ($P < 0.001$). In contrast, the number of half-cystine groups of transferase A

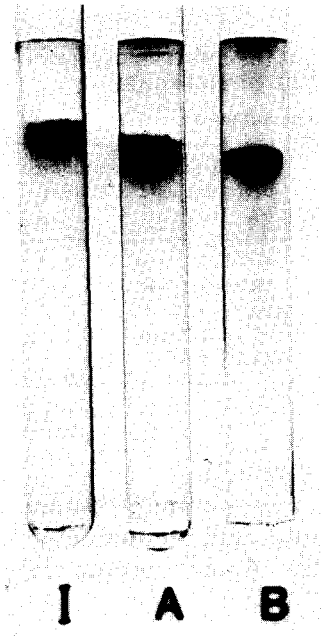

Fig. 2. Band pattern of the various forms of mitochondrial acetyl-CoA acetyltransferase on polyacrylamide gel electrophoresis. From left to right: 10 μg of transferases I, A and B, respectively.

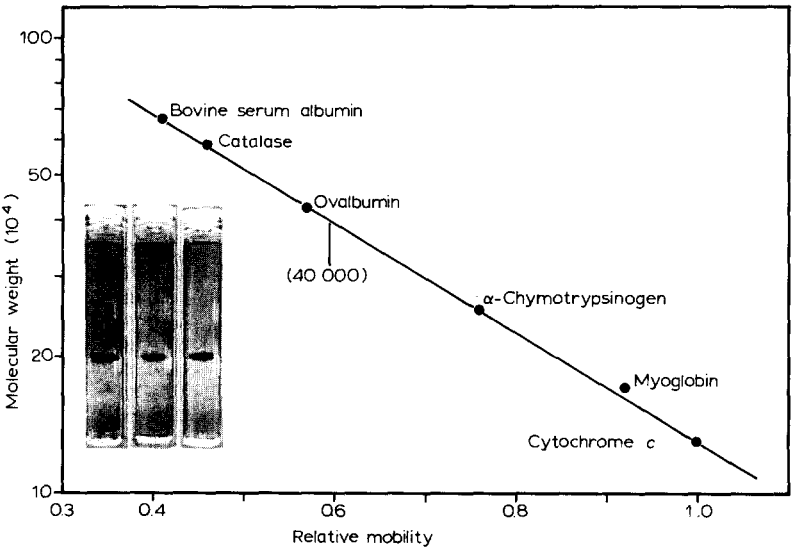

Fig. 3. Determination of the subunit molecular weights of mitochondrial acetyl-CoA acetyltransferases I, A and B by SDS-polyacrylamide (8%) gel electrophoresis. Reference proteins used: bovine serum albumin (66 000), catalase (60 000) ovalbumin (43 000), chymotrypsinogen (25 700), myoglobulin (17 200) and horse heart cytochrome *c* (13 370).

TABLE I

ACCESSIBLE AND TOTAL SULFHYDRYL GROUPS AND TOTAL HALF-CYSTINE CONTENT OF THE I, A AND B FORMS OF MITOCHONDRIAL ACETYL-CoA ACETYLTRANSFERASE

Results are expresed as means ± S.E. with the number of experiments in parentheses.

Transferases	Mol sulfhydryl per mol enzyme		Cysteic acid content after performic acid oxidation
	Without SDS	With SDS	
I	—	10.9 ± 1.1 (3)	—
A	13.4 ± 1.8 (5)	20.5 ± 2.9 (8)	18.4 ± 1.1 (4) (20.1 2.1) *
B	13.6 ± 1.7 (6)	14.3 ± 1.6 (10)	21.1 ± 1.7 (4) (22.9 1.8)

* Means ± S.E. obtained by assuming a recovery of 92% [15].

did not significantly differ from that of transferase B (Table I).

The amino acid composition of the transferases A and B are presented in Table II. Both forms are composed of about 1432 amino acid residues. Despite the finding of small differences (<0.8%) in the number of some specific amino

TABLE II

AMINO ACID COMPOSITION AND N-TERMINAL RESIDUE OF THE TWO MAIN DIFFERENT FORMS OF THE MITOCHONDRIAL ACETYL-CoA ACETYLTRANSFERASE FROM BOVINE LIVER

Samples of each enzyme form were hydrolyzed for 24, 48 and 72 h. The average of the three times (±S.E.) is given with the exception for serine, threonine, tyrosine, tryptophan and cysteine.

Amino acid	Number of residues per mol enzyme for a molecular weight of 150 000		Difference (A—B)	
	Transferase A	Transferase B	Number	Percent residues per 100 residues
Asp	130.7 ± 4.9	137.3 ± 3.5	6.6	0.35
Thr	75.7 ± 1.7 *	73.2 ± 1.2 *	2.3	0.30
Ser	89.6 ± 2.3 *	81.9 ± 0.9 *	7.7	0.56
Glu	116.8 ± 1.6	124.4 ± 3.3	7.6	0.48
Pro	64.2 ± 5.2	61.9 ± 1.1	2.3	0.20
Gly	120.2 ± 5.4	124.0 ± 3.6	3.9	0.23
Ala	159.7 ± 2.5	171.6 ± 4.2	11.9	0.73
Val	133.3 ± 2.6	142.9 ± 4.2	9.2	0.57
Met	48.5 ± 1.5	51.8 ± 1.3	3.3	0.20
Ile	84.9 ± 3.2	75.7 ± 3.3	10.1	0.74
Leu	111.0 ± 4.4	102.1 ± 4.6	9.2	0.68
Tyr	36.1 ± 1.0 *	35.6 ± 3.2 *	0.1	0
Phe	28.1 ± 3.2	27.0 ± 1.3	1.1	0.09
His	19.6 ± 0.5	23.5 ± 1.4	3.9	0.23
Lys	132.8 ± 2.1	123.5 ± 3.1	9.3	0.70
Arg	40.3 ± 1.6	43.8 ± 1.5	3.5	0.24
Cys	20.0 ± 1.8 **	22.9 ± 0.9 **	2.9	0.20
Trp	16.0 ± 2.1	14.0 ± 1.8	2.0	0.14
Total number of residues	1428.5	1437.2	96.9	Δ% : 6.64
N-Terminal residue	Arg	Arg		

* Values were extrapolated to zero time, based upon data obtained after 24 h hydrolysis, assuming first-order kinetics.

** Determined as cysteic acid after performic acid oxidation, assuming 92% recovery.

TABLE III

MOLECULAR PROPERTIES OF THE VARIOUS FORMS OF THE MITOCHONDRIAL ACETYL-CoA ACETYLTRANSFERASES

For a comparison data of the transferases I, A and B were taken from Table I.

Properties	Acetyl-CoA acetyltransferases					
	I	A	B	p I 6.9 form	p I 7.5 form	pI 8.8 form
Molecular weight						
by sedimentation analysis	—	154 600	147 600	—	—	—
by gel filtration	155 000	150 000	152 000	150 000	157 000	151 000
Subunit molecular weight	40 000	40 000	40 000	39 000	40 000	39 000
Free sulfhydryl (mol of sulfhydryl/mol enzyme) *						
accessible	—	13.4 ±1.8 (5)	13.6 ±1.7 (6)	—	—	14.0 ±0.8 (5)
total	10.9 ±1.1 (3)	20.5 ±2.9 (8)	14.3 ±1.6 (10)	10.7 ±1.1 (5)	18.4 ±1.9 (5)	13.6 ±2.7 (5)
Isoelectric points	6.8	6.9	—	6.9	—	—
	7.4	7.5	—	—	7.5	—
	8.8	8.8	8.8	—	—	8.8

* Values are given as means ± S.E. with the number of experiments in parentheses.

acid residues between the two forms the transferases A and B appear remarkably similar, if not identical in their amino acid composition. In addition arginine has been identified as NH_2-terminal residue of both forms.

Molecular and subunit molecular weight

The molecular weights of the transferases I, A and B were estimated by different methods. In gel filtration enzymic activities were eluted as single symmetrical peaks and the relative mobilities of transferases I, A and B corresponded to molecular weights of 155 000, 150 000 and 152 000, respectively (Tabel III). The molecular weights of the transferases A and B, as determined by sedimentation equilibrium experiments, agree well with the values obtained from gel filtration (Table III). The sedimentation coefficients (s_{20}) were 7.48 S and 7.72 S for these two forms, respectively. Each of the SDS-dissociated transferases I, A and B gave rise to a single-stained protein band in polyacrylamide gel electrophoresis and showed mobilities which corresponded to a subunit molecular weight of 40 000 for all forms (Fig. 3). Thus, the multiple native forms of the acetyl-CoA acetyltransferase appear to be tetrameres composed of four similar subunits.

Isoelectric focusing

To characterize the different enzyme forms I, A and B in terms of the overall net charge the enzymes were subjected to isoelectric focusing in an ampholyte pH-gradient from 6 to 10 (Fig. 4). As was established in several experiments, transferase A appeared in three forms with isoelectric points of 6.9 ± 0.1, 7.5 ± 0.1 and 8.8 ± 0.2 (in the following, designated as the p*I* 6.9, p*I* 7.5 and

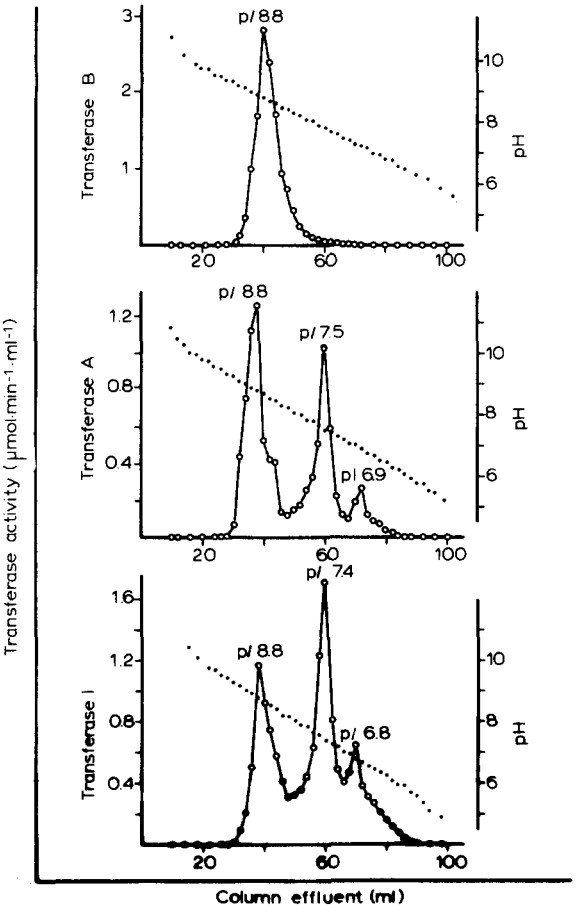

Fig. 4. Isoelectric focusing of mitochondrial acetyl-CoA acetyltransferases I, A and B. Lower part: transferase I (38 units); middle part: transferase A (25 units) and upper part: transferase B (24 units). Isoelectric focusing was conducted for 72 h at 4°C. The column contents were collected in fractions of 2.0 ml; •, pH at 4°C; ○, enzyme activity in the direction of acetoacetyl-CoA cleavage (recovery of enzyme activity about 70%).

p*I* 8.8 forms, respectively). The distribution pattern of enzymatic activities was in favour of the p*I* 8.8 form. Transferase I also revealed multiple forms with isoelectric points of 6.8, 7.4 and 8.8, but the enzymatic activity was in favour of the p*I* 7.4 form. On the contrary transferase B showed only one activity peak with an isoelectric point of 8.8 ± 0.1.

Molecular properties of the various pI forms

The molecular weights of the three p*I* forms of the transferases I, A and B, as estimated by gel filtration on standardized columns of Sephadex G-200, were in the range of 150 000—157 000 (Table III). Each of the three p*I* forms displayed only one band on SDS-polyacrylamide gel electrophoresis with a molecular weight of 39 000, suggesting that the various p*I* forms are composed of four subunits. Analysis of the sulfhydryls of the p*I* forms indicated the pres-

ence of 10.7, 18.4 and 13.6 sulfhydryl groups per enzyme molecule of the p*I* 6.9, 7.5 and 8.8 forms, respectively (Table III).

Multiplicity of forms of mitochondrial acetyl-CoA acetyltransferase

Transformation of the transferases I and A into different three forms as found during isoelectric focusing was also observed after rechromatography of the various p*I* forms on phosphocellulose. Thus the p*I* 6.9 and 7.5 forms yielded three and two forms, respectively, when rechromatographed on phosphocellulose; the p*I* 6.9 form, resulted in three fractions, which corresponded to the transferases I, A and B (Fig. 1); the p*I* 7.5 form was redistributed into two fractions, corresponding to transferases A and B. In addition, when the transferases I and A were rechromatographed on phosphocellulose, again these proteins were eluted in three forms. Moreover, with respect to the elution

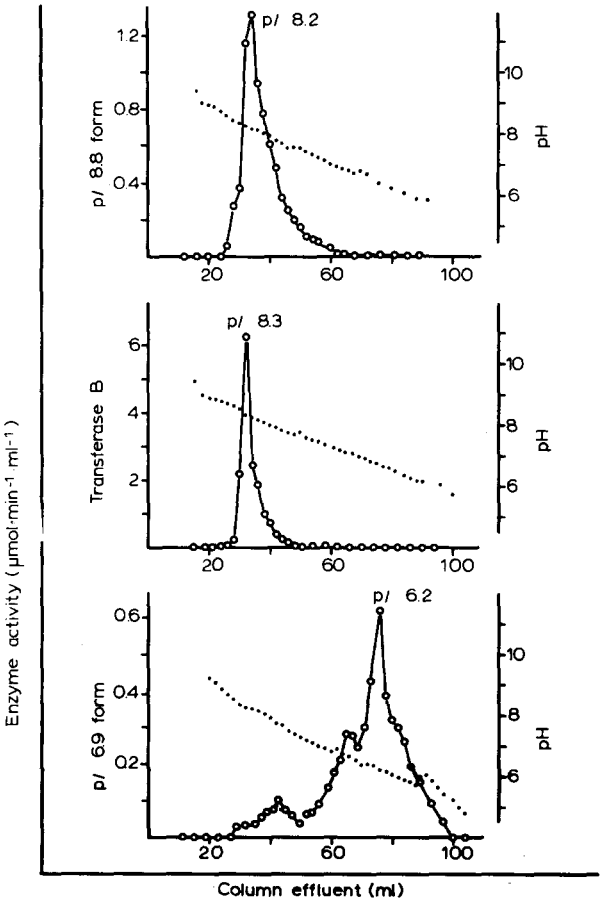

Fig. 5. Isoelectric focusing of transferase B and the p*I* 8.8 and 6.9 forms after incubation (90 min, 30°C) in presence of dithioerythritol (50 mM). Upper part: p*I* 8.8 form (12 units); middle part: transferase B (35 units); lower part: p*I* 6.9 form (8 units). Isoelectric focusing was conducted for 72 h at 4°C. The column contents were collected in fractions of 2 ml; •, pH at 4°C; ○, enzyme activity in direction of acetoacetyl-CoA cleavage (recovery of enzyme activity about 85%).

TABLE IV

EFFECT OF DITHIOERYTHRITOL ON SULFHYDRYL GROUPS AND ISOELECTRIC POINTS OF VARIOUS FORMS OF MITOCHONDRIAL ACETYL-CoA ACETYLTRANSFERASE

The protein solutions were incubated for 90 min at 30°C in presence of 50 mM dithioerythritol and subsequently subjected to isoelectric focusing in presence of dithioerythritol or, after removal of the reducing agent to sulfhydryl group analysis. Results are expressed as mol of sulfhydryl per mol enzyme.

Incubation in presence of dithioerythritol	pI form		Transferase B
	6.9	8.8	
Free sulfhydryls	16.9	17.6 ± 1.0 (3) *	20.2 ± 2.2 (4) *
Isoelectric points	6.2	8.2	8.3

* Values are means ± S.E. Number of experiments are given in parentheses.

positions from phosphocellulose and the sulfhydryl group content, the three forms obtained by rechromatography on phosphocellulose corresponded with transferases I, A and B, respectively (Fig. 1 and Table III).

Transferase B and the pI 8.8 form were rechromatographed on phosphocellulose as a single peak of activity of the same position as that for the original peak of transferase B (Fig. 1).

A change in the redox state of the enzyme protein may be responsible for the appearance of the multiple enzyme forms. Thus a decrease in the number of total sulfhydryl groups per molecule could at least theoretically shift the isoelectric point from 7.5 to 8.8. Reduction by dithioerythritol of the p*I* 8.8 form or of transferase B led indeed to an increase in the sulfhydryl groups per enzyme molecule to values of the p*I* 7.5 form or transferase A without loss of enzymatic activity; however a subsequent isoelectric focusing in presence of the reducing agent revealed p*I* values of only 8.2 and 8.3, respectively (Table IV, Fig. 5, upper and middle part). Similary the p*I* value of the p*I* 6.9 form decreased to a value of 6.2, after reductive treatment; in addition two small activity peaks indicating p*I* values of 6.7 and 7.7 could be demonstrated (Table IV, Fig. 10, lower part).

Discussion

The activity of the bovine liver mitochondrial acetyl-CoA acetyltransferase is associated with different protein forms, which differ in their kinetic and regulatory properties which may be of physiological significance [10,20,21]. This report presents data about some molecular properties of the multiple forms of this enzyme.

The transferases I, A and B are of similar size and consist of four similar subunits. Total free sulfhydryl groups, molecular weights, subunit molecular weights and the affinities for cation exchanger suggest, that the transferases I, A and B are identical with the p*I* 6.9, 7.5, 8.8 forms, respectively. In particular, on the basis of the stability of the protein states as well as of the redox states, some evidence has been found for an identical protein state of transferase B and the p*I* 8.8 form. Thus sulfhydryl groups of transferase B and of the p*I* 8.8 form were elevated to an almost fully reduced state upon reductive treatment

and both revealed an almost identical decrease in isoelectric points. Although the redox states of the two forms then correspond to those of transferase A or the p*I* 7.5 form, both forms differ with respect to the isoelectric points and the ability to be transformed into multiple forms. Thereby it is obvious that reduced transferase B or the reduced p*I* 8.8 form represents a state of the enzyme protein which is different from that of transferase A or the p*I* 7.5 form. As far as transferases I and A are concerned, similar multiple forms exist, although transferases I and A differ in their redox states. It is therefore concluded that changes in the redox state may not cause the observed multiplicity.

Evidence has been obtained that transferase A represents the main state of the mitochondrial acetyl-CoA acetyltransferase which is able to be spontaneously transformed into forms which differ in their isoelectric points and in their redox state. Transferase B appears to be a stable enzyme form, it elutes as a single activity peak upon rechromatography on phosphocellulose, gel electrophoresis and isoelectric focusing. Transferases A and B appear to be remarkably similar in amino acid compositions except for possible differences in amide nitrogen. Also immunochemical studies performed on transferases A and B of rat liver mitochondrial acetyl-CoA acetyltransferase revealed immunochemical cross-reactivity between these two forms (Huth, W. and Schwabe, D., unpublished). The origin of the multiple forms is not yet known. Our data suggests, that the transformation of mitochondrial acetyl-CoA acetyltransferase into multiple forms, represented by charge differences, may have been either preformed in vivo by post-translational modifications or introduced during working up through chemical modifications. Chemical modification by deamidation of glutamine and asparagine may occur [22,23] but this reaction cannot be the only mechanism of a process giving rise to these multiple forms. According to Middleton [8,24] transferase A is present in tissues capable of ketogenesis but it has very low or undetectable activity in extrahepatic tissues. The activity of the mitochondrial acetyl-CoA acetyltransferase is elevated in livers from starved rats, thus possibly increasing the rate of ketogenesis [25].

The results of this study indicate the existence of three forms of the mitochondrial acetyl-CoA acetyltransferase namely I, A and B, which differ in their redox states, isoelectric points and in their ability to be spontaneously transformed into various forms. The exact relationships between these multiple forms remain to be further analyzed.

Acknowledgements

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