Purification and Properties of Succinyl-Coenzyme A-3-Oxo Acid Coenzyme A-Transferase from Sheep Kidney

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(Received 12 December 1977)

CoA-transferase (succinyl-CoA-3-oxo acid CoA-transferase, EC 2.8.3.5) isolated from sheep kidney was purified to homogeneity. The purified enzyme has a specific activity of approx. 200 units/mg. A mol.wt. of 110000 was obtained by gel filtration on Sephadex G-200, and a lower mol.wt. of 102000 was determined by analytical ultracentrifugation. A sedimentation coefficient ($s_{20,w}^0$) of 5.6S was also determined. A subunit mol.wt. of 56000 was obtained by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Isoelectric focusing of sheep kidney extracts indicated the presence of a single band of CoA-transferase activity with pI9.0. However, isoelectric focusing of purified CoA-transferase showed the presence of two peaks of CoA-transferase activity with pI values of 8.7 and 8.4, suggesting the presence of proteolytic activity during purification. Evidence for sheep kidney CoA-transferase being a dimer of two identical subunits has been obtained from sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, the amino acid composition, peptide 'mapping' and N-terminal analysis.

CoA-transferase (succinyl*-CoA-3-oxo acid CoA-transferase, EC 2.8.3.5) catalyses the reaction:

CoA-transferase from mammalian sources has previously only been purified to homogeneity from pig heart (Edwards *et al.*, 1973; Hersh & Jencks, 1967*a,b*), although enzymes exhibiting similar functional properties have been purified from bacterial sources, including *Escherichia coli* (Sramek & Ferman, 1975) and *Peptostreptococcus elsdenii* (Schulman & Valentino, 1976).

CoA-transferase from pig heart has been studied extensively (Stern *et al.*, 1956; Hersh & Jencks, 1967*a,b*; Solomon & Jencks, 1969; White & Jencks, 1976*a,b*; White *et al.*, 1976) and these studies have determined the amino acid composition, kinetic mechanism and kinetic properties of the active site. These studies have principally concerned a kinetic analysis of the enzyme, and no structural studies have been attempted, as lengthy purification procedures and low yields would make any extensive structural study of pig heart CoA-transferase impractical.

Sheep kidney has been established as one of the richest sources of CoA-transferase (Williamson *et al.*, 1971), containing at least 35 units/g wet wt. of tissue, compared with 12 units/g in pig heart (Edwards *et al.*, 1973).

Abbreviation used: dansyl, 5-dimethylaminonaphthalene-1-sulphonyl.

* Refers to 3-carboxypropionyl-CoA.

The present paper describes a procedure for purifying sheep kidney CoA-transferase to homogeneity, with yields that make a structural study feasible. The results of studies on the physical properties of this CoA-transferase are also reported.

Experimental

Materials

Chemicals and chromatographic media were obtained from the following suppliers: CoA (grade I, 85–90% pure), marker proteins (catalase, aldolase, lactate dehydrogenase, malate dehydrogenase, cytochrome *c*, bovine serum albumin, glutamate dehydrogenase, ovalbumin, alcohol dehydrogenase and lysozyme) and poly(ethylene glycol) from Sigma Chemical Co., St. Louis, MO, U.S.A.; Ampholines from LKB-Produkter, Bromma, Sweden; Sephadex G-25, G-200 and Blue Sepharose CL-6B from Pharmacia Fine Chemicals, Uppsala, Sweden; Bio-Rex 70 (200–400 mesh) from Bio-Rad Laboratories, Richmond, CA, U.S.A.; diketene from Fluka, Buchs, Switzerland; DEAE-cellulose (DE-32) and 3MM chromatography paper from Whatman Biochemicals, Maidstone, Kent, U.K.; trypsin from Worthington Biochemical Corp., Freehold, NJ, U.S.A. All other reagents were of the highest purity readily available. Glass-distilled deionized water was used for rinsing all glassware and for the preparation of buffers. Acetoacetyl-CoA was prepared by the method of Wieland & Rueff (1953).

Sheep kidney, heart and brain, and pig heart,

kidney and brain, obtained from the State Abattoirs, Homebush, N.S.W., Australia, were transported to the laboratory in ice, where they were stripped of fat, washed in deionized water and then stored at -20°C .

Isoelectric focusing

Isoelectric focusing was performed at 4°C in a glycerol gradient in a $1.5\text{ cm} \times 30\text{ cm}$ Perspex column (total volume 55 ml). Ampholines of wide pH range (pH 3.5–10) or narrow pH range (pH 7–10) were used. Samples for isoelectric focusing were either pure CoA-transferase or tissue extracts.

Tissue extracts were prepared by homogenization of tissue in 3 vol. of 0.4 M-KCl containing 20% (v/v) ethanol and centrifugation at $10000g$ for 20 min at 4°C . The supernatants (approx. 10 ml) were dialysed against 5 litres of distilled deionized water for 2 h. Samples (4.2 ml) were added to the centre of a linear glycerol gradient (20–60%, v/v) (Vesterberg, 1971). Electrofocusing was performed for 72 h at 1200 V and 4°C . Fractions (1.5 ml) were collected and assayed for CoA-transferase activity. The pH of the fractions was measured with a Pye model 290 pH-meter (fitted with a Philips CA14/02 semi-micro-electrode).

Purification

Frozen sheep kidney (200 g) in 600 ml of 0.4 M-KCl containing 20% (v/v) ethanol was homogenized in a blender for 3 min at 4°C . The homogenate was centrifuged for 20 min at $10000g$, 4°C , and the resulting supernatant was dialysed for two 8 h periods at 4°C against 10 litres of $10\text{ mM-K}_2\text{HPO}_4$, pH 7.0, containing 1 mM-EDTA . After dialysis the extract was adjusted to 45% saturation with $(\text{NH}_4)_2\text{SO}_4$ at 4°C by the addition of 278 g of $(\text{NH}_4)_2\text{SO}_4$ /litre of extract and stirred for 30 min at 4°C . The precipitate that formed was discarded after centrifugation (20 min at $10000g$, 4°C). The supernatant was adjusted to 60% saturation with $(\text{NH}_4)_2\text{SO}_4$ at 4°C by the addition of 98 g of $(\text{NH}_4)_2\text{SO}_4$ /litre and was stirred for 30 min at 4°C . The precipitate was collected by centrifugation as above, and resuspended in buffer I ($5\text{ mM-K}_2\text{HPO}_4$, pH 7.5, containing 1 mM-EDTA) and then desalted by gel filtration on a Sephadex G-25 column ($5\text{ cm} \times 100\text{ cm}$) previously equilibrated with buffer I.

All column-chromatography steps in the purification of CoA-transferase were performed at room temperature (20 – 30°C). The void-volume effluent was adjusted to pH 8 by the addition of 0.1 M-KOH and chromatographed on DEAE-cellulose (DE-32) ($5\text{ cm} \times 20\text{ cm}$ column), equilibrated with $5\text{ mM-K}_2\text{HPO}_4$, pH 8, containing 1 mM-EDTA (buffer II). The protein was applied to the DEAE-cellulose, and unbound material was eluted from the column with buffer II. This unbound fraction contained approx. 90% of the CoA-transferase activity that was applied to the column. The pH of this unbound fraction was adjusted to pH 7.5 by the addition of 0.1 M-acetic acid .

The CoA-transferase could then be purified by chromatography on Bio-Rex 70 (a weakly acidic resin containing carboxylic acid-exchange groups on an acrylic polymer lattice). Protein was applied to the Bio-Rex 70 ($4\text{ cm} \times 30\text{ cm}$ column), equilibrated with buffer I. Chromatography was performed starting with 400 ml of buffer I containing 0.05 M-KCl and developed with an 800 ml linear gradient of 0.05 – 0.15 M-KCl in buffer I. Fractions containing CoA-transferase activity were pooled, concentrated by the addition of 560 g of $(\text{NH}_4)_2\text{SO}_4$ /litre (80% saturation), stirred for 30 min at 4°C , and centrifuged at $10000g$ for 20 min. The pellet was resuspended in buffer I and then dialysed against 10 litres of buffer I for 9 h at 4°C . The CoA-transferase was then further purified by chromatography on Blue Sepharose (Cibacron Blue F3G-A covalently attached to the cross-linked agarose-gel-Sepharose CL-6B). The Blue Sepharose (in a $1.5\text{ cm} \times 20\text{ cm}$ column) was equilibrated with buffer I. Chromatography was performed with buffer I and developed with a linear salt gradient (300 ml) of 0 – 0.25 M-KCl in buffer I. CoA-transferase was eluted with approx. 0.14 M-KCl .

Fractions containing CoA-transferase activity from the Blue Sepharose were dialysed against buffer I (10 litres at 4°C) for 9 h, and concentrated to 1 mg/ml by dialysis against a saturated solution of poly(ethylene glycol) at 4°C . The protein was then stored at -20°C .

Enzyme assay

CoA-transferase was assayed spectrophotometrically by following the disappearance of acetoacetyl-CoA at 303 nm (Edwards *et al.*, 1973). A unit of CoA-transferase activity catalyses the conversion of $1\text{ }\mu\text{mol}$ of acetoacetyl-CoA into acetoacetate per min at 30°C and pH 8.1 under the conditions of the assay.

Protein concentrations were measured spectrophotometrically (Kalckar, 1947) or by the microbiuret method (Itzhaki & Gill, 1964), with bovine serum albumin as a standard.

Polyacrylamide-gel electrophoresis

The purity of the CoA-transferase preparations was examined by gel electrophoresis at pH 4.5 (Gabriel, 1971) and pH 9.5 on 5% polyacrylamide gels. The pH 9.5 polyacrylamide gels were prepared as described by Gabriel (1971) for pH 8.3 gels, except that buffers were adjusted to pH 9.5 rather than pH 8.3.

Molecular-weight determination

Molecular weights were determined by the method of Andrews (1965), by using Sephadex G-200 ($1.5\text{ cm} \times 100\text{ cm}$ column). Sephadex G-200 was hydrated and equilibrated with $100\text{ mM-KH}_2\text{PO}_4$, pH 6.5. Marker proteins [catalase (230000), aldolase (150000), lactate dehydrogenase (140000), malate dehydrogenase (60000) and cytochrome *c* (12400)], used as mole-

cular-weight standards, were detected by enzyme activity by using standard spectrophotometric assay procedures, or by their A_{280} .

Molecular-weight determinations of CoA-transferase subunits were performed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Weber & Osborn, 1969). Samples were prepared by the method of Pringle (1970). Protein standards used were bovine serum albumin (68000), glutamate dehydrogenase (53000), ovalbumin (43000), alcohol dehydrogenase (37000) and lysozyme (14300).

Molecular weights were calculated from the linear portion of a plot of $\log$ (mol.wt.) against elution volume or electrophoretic mobility for gel filtration and sodium dodecyl sulphate/polyacrylamide-gel electrophoresis respectively (Andrews, 1965; Weber & Osborn, 1969).

Ultracentrifugation

The molecular weight and sedimentation coefficient were determined in a Spinco model E analytical ultracentrifuge with an AN.F rotor at room temperature (19–20°C). Protein samples were equilibrated with 50 mM-KH₂PO₄, pH 6.5, by gel filtration through a Sephadex G-25 column (0.9 cm × 15 cm) or by dialysis over a period of 24 h.

For molecular-weight determinations, centrifugation was performed at 60000 rev./min at protein concentrations of 0.5, 1.0 and 2.0 mg/ml. Interference optics were used to monitor protein migration.

The sedimentation coefficient was determined by centrifugation at 18000 rev./min at protein concentrations of 1, 4 and 7 mg/ml. Protein migration was monitored by the use of schlieren optics.

All calculations were performed by the methods described by Chervenka (1969) and corrected for 20°C. A partial specific volume of 0.75 ml/g was estimated from the amino acid composition of sheep kidney CoA-transferase (Chervenka, 1969).

Amino acid analysis

Three samples each containing 5 mg of pure CoA-transferase (specific activity 200 units/mg) were hydrolysed for 24, 48 or 72 h at 110°C in 6 M-HCl containing 1% (w/v) phenol (Sanger & Thompson, 1963) in sealed evacuated tubes. The amino acid compositions of the total acid hydrolysates were analysed with a Beckman model 121-M amino acid analyser.

Values for threonine and serine were extrapolated to zero time to allow for decomposition of these amino acids during hydrolysis (Hill, 1965). Tryptophan was determined spectrophotometrically by the method of Beavan & Holiday (1952) by using a Cary 118 spectrophotometer. After pretreatment of the respective samples, cysteine and cystine were determined by reaction with 5',5'-dithiobis-(2-nitrobenzoic acid) (Ellman, 1959).

For the determination of cysteine, CoA-transferase was first denatured by incubation in 8 M-urea at room temperature. Cystine was determined by using CoA-transferase samples that were first denatured in the presence of 8 M-urea and 0.1 M-2-mercaptoethanol to form cysteine. Excess 2-mercaptoethanol was removed by gel filtration on a Sephadex G-25 column (0.9 cm × 6 cm) equilibrated with 8 M-urea.

Dansylations were performed by the method described by Air & Thompson (1969), except that 0.1 M-triethanolamine was used in place of 0.2 M-NaHCO₃ buffer.

Peptide 'mapping' was performed by the method of Thompson *et al.* (1969).

Results

Isoelectric focusing

The possibility of multiple forms of CoA-transferase has been reported in pig heart (White & Jencks, 1976b) and rat kidney (Fenselau & Wallis, 1974). To determine whether there were multiple forms of the enzyme in sheep kidney, a KCl/ethanol extract was subjected to isoelectric focusing. A single peak of activity was obtained, which corresponded to a pI value of 9.0 (Fig. 1). For comparison, isoelectric focusing was also used to study extracts from sheep heart and brain and pig heart, brain and kidney. A single enzyme form was present in each of these tissues, and the results are summarized in Table 1.

Isoelectric focusing of purified CoA-transferase

Fig. 1. Isoelectric focusing of sheep kidney CoA-transferase. Isoelectric focusing was performed on a KCl/ethanol extract of sheep kidney after gel filtration [on a column of Sephadex G-25 equilibrated with 20% (v/v) glycerol]. Electrofocusing was performed in a 55 ml column for 72 h at 1200 V, 4°C. Ampholines pH 7–10 range were used: 1.5 ml fractions were collected and 10 μ l samples were assayed for CoA-transferase activity (○) by the procedure described by Edwards *et al.* (1973). ●, pH.

from sheep kidney resulted in the separation of two peaks of CoA-transferase activity (Fig. 2) with pI values of 8.7 and 8.4.

Purification

The purification procedure, which makes use of the unusually high pI of sheep kidney CoA-transferase, is summarized in Table 2. A typical elution profile obtained from Bio-Rex 70 is shown in Fig. 3.

Although the Blue Sepharose column chromatography did not increase the specific activity of the CoA-transferase preparation, its inclusion was found to be necessary when enzyme preparations were to be stored. Methods of storage were not investigated in detail, but when this step was not included in the purification procedure CoA-transferase preparations lost approx. 70% (final specific activity = 60 units/mg) of their original activity within 1 month of storage at -20°C . Analysis of these fractions by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis revealed the presence of multiple bands, which may be the result of minor contamination by proteinase activity. The inclusion of the Blue Sepharose step prevented this loss of activity, and analysis of samples

stored at -20°C , by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, showed the presence of one major band. Other methods of storage, such as storing the enzyme as an $(\text{NH}_4)_2\text{SO}_4$ suspension at 4°C or -20°C , were not successful and resulted in the rapid loss of CoA-transferase activity.

Polyacrylamide-gel electrophoresis at pH 4.5 and 9.5 showed the presence of only one band in enzyme preparations with a specific activity of 200 units/mg, and ultracentrifugation of purified CoA-transferase resulted in a single symmetrical peak. On the basis of these results, CoA-transferase preparations with specific activities of 200 units/mg were considered to be pure.

Table 1. *Isoelectric focusing of sheep and pig tissues*
Tissues were homogenized in 0.4M-KCl/20% (v/v) ethanol and desalted by using gel filtration on Sephadex G-25. Extracts were then subjected to isoelectric focusing for 72 h at 1200 V, 4°C . Wide-range Ampholines (pH 3.5–10) were used for all extracts. Narrow-range Ampholines (pH 7–10) were also used for sheep kidney samples. Values given are the pI values obtained for CoA-transferase from these tissue extracts. Values in parentheses are for purified proteins.

	Heart	Kidney	BRAIN
Pig	5.5 (6.5*, 6.2* and 5.93*)	5.5	4.8
Sheep	8.2	9 (8.7 and 8.4)	6.3

* These values were obtained from White & Jencks (1976b).

Fig. 2. *Isoelectric focusing of purified sheep kidney CoA-transferase*

Isoelectric focusing was performed on purified preparations of sheep kidney CoA-transferase. Isoelectric focusing was performed in a 55 ml column for 72 h at 1200 V, 4°C . Ampholines pH 7–10 range were used: 1.5 ml fractions were collected and 10 μl samples were assayed for CoA-transferase activity (○) by the method described by Edwards *et al.* (1973). ●, pH.

Table 2. *Summary of the purification of sheep kidney CoA-transferase*

The purification procedure is described in the Experimental section. All assays were measured by the disappearance of acetoacetyl-CoA. Protein was measured spectrophotometrically (Kalckar, 1947). Protein determined by the microbiuret method (Itzhaki & Gill, 1964) gave a value approx. 3% higher, resulting in a specific activity of 220 units/mg for the purified enzyme from Blue Sepharose.

Fraction	Volume (ml)	Activity (units/ml)	Total activity (units)	Protein (mg/ml)	Specific activity (units/mg)	Purification (fold)	Yield (%)
Homogenate	630	18.2	11 500	18.4	0.99	1	100
45–60%-satd. $(\text{NH}_4)_2\text{SO}_4$	43	240	10 300	54.6	4.4	4.4	90
DEAE-cellulose	200	43.8	8760	1.5	29.2	29.5	76
Bio-Rex 70	90	49.7	4470	0.22	226	228	39
Blue Sepharose	150	29.3	4400	0.13	225	228	38
Poly(ethylene glycol) concentrated	20	200	4000	1.0	200	202	35

Fig. 3. Elution profile of CoA-transferase from Bio-Rex 70

CoA-transferase purified as described in the Experimental section was loaded on a column (4 cm $\times$ 30 cm) packed with Bio-Rex 70, equilibrated with 5 mM- K_2HPO_4 (pH 7.5)/1 mM-EDTA. The column was washed with the above buffer containing 0.05 M-KCl, until all unbound material was washed from the column. CoA-transferase was eluted with a linear gradient of 0.05–0.15 M-KCl in 5 mM- K_2HPO_4 (pH 7.5)/1 mM-EDTA; 20 ml fractions were collected and fractions containing CoA-transferase activity were pooled. Δ , KCl concentration; $\bullet$, A_{280} ; $\circ$, CoA-transferase activity.

Table 3. Amino acid composition of sheep kidney CoA-transferase

Pure CoA-transferase was hydrolysed as described in the Experimental section. Values given are for 24, 48 and 72 h hydrolyses in 6 M-HCl *in vacuo*. Residues are calculated as mol/mol of CoA-transferase, based on a mol.wt. of 102 000.

Amino acid	24 h	48 h	72 h	Average
Lysine	78.3	69.8	72.1	73.4
Histidine	16.8	16.1	16.2	16.4
Arginine	37.3	34.1	34.2	35.2
Aspartate	78.3	82.4	83.6	81.5*
Threonine	—	—	—	48†
Serine	—	—	—	30†
Glutamate	81.1	80.6	81.1	81*
Proline	41.0	44.8	44.2	43
Glycine	94.2	91.4	93.7	93
Alanine	76.5	79.7	77.5	78
Cysteine	—	—	—	8‡
Valine	82.1	86.0	88.3	85.5
Methionine	23.4	23.4	23.4	23.4
Isoleucine	64.3	64.5	63.1	64
Leucine	85.8	86.0	84.7	85.5
Tyrosine	18.7	19.7	18.0	18.8
Phenylalanine	30.6	32.3	32.4	31.8
Cystine	—	—	—	0‡
Tryptophan	—	—	—	3.4§

* Glutamate and aspartate refer to Glx and Asx respectively.

† Values were extrapolated to zero time to correct for decomposition during hydrolysis.

‡ Values were measured by using 5,5'-dithiobis-(2-nitrobenzoic acid) as described in the Experimental section.

§ Determined spectrophotometrically by the method of Beavan & Holiday (1952).

Molecular-weight determinations

The molecular weight of CoA-transferase isolated from sheep kidney was estimated to be 110 000 by gel-filtration chromatography on Sephadex G-200. A lower mol.wt. of 102 000 was obtained for CoA-transferase by analytical ultracentrifugation. A sedimentation coefficient $s_{20,w}^0$ of 5.6 S was also obtained, by analytical ultracentrifugation, for CoA-transferase. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of samples stored at -20°C , after Blue Sepharose chromatography, detected the presence of a single major band corresponding to a mol.wt. of 56 000. However, if samples were stored before Blue Sepharose chromatography, two additional major bands in the CoA-transferase preparations could be detected. These major bands had mol.wts. of 24 000 and 27 000.

Amino acid composition of CoA-transferase

The amino acid composition of the sheep kidney CoA-transferase, based on a mol.wt. of 102 000, is presented in Table 3. Enzymic digestion of CoA-transferase with trypsin and subsequent peptide 'mapping' of the digest resulted in the separation of approx. 43 ninhydrin-positive tryptic peptides. *N*-Terminal amino acid analysis by the dansyl chloride method on samples of pure CoA-transferase did not detect the presence of a free *N*-terminal amino acid.

Discussion

The purification procedure described for sheep kidney CoA-transferase takes advantage of the unusually high isoelectric point (pI 9) of this enzyme.

Pig heart CoA-transferase has been successfully purified by substrate elution with acetoacetyl-CoA from a cellulose phosphate cation-exchange column at pH 6.5 (Edwards *et al.*, 1973). The sheep kidney CoA-transferase bound strongly to cellulose phosphate at pH 6.5, but attempts at subsequent substrate elution with acetoacetyl-CoA were not successful. At higher pH values (pH 8.5–9.0), at which sheep kidney CoA-transferase binds weakly to cellulose phosphate, attempts at substrate elution gave non-reproducible results, probably as a result of the instability of acetoacetyl-CoA and/or enzyme-CoA at these pH values.

Isoelectric focusing of KCl/ethanol extracts from sheep kidney resulted in a single peak of CoA-transferase activity, as did extracts from sheep heart and brain and pig heart, brain and kidney. Previous reports by White & Jencks (1976b) have suggested that CoA-transferase from pig heart is present as three kinetically homogeneous isoenzymes. Fenselau & Wallis (1974) have reported that two isoenzymes of CoA-transferase may be present in rat kidney. These multiple forms obtained by isoelectric focusing of purified or partially purified CoA-transferase could be the result of limited proteolysis of the CoA-transferase. Fenselau & Wallis (1976) have shown that limited proteolysis of rat muscle CoA-transferase, with trypsin and chymotrypsin, shifted the pI of CoA-transferase from 7.4 to 6.9 and 6.6 respectively. In the present study, isoelectric focusing of purified CoA-transferase resulted in the separation of two peaks of CoA-transferase activity, and sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of CoA-transferase stored before Blue Sepharose chromatography shows the presence of more than one band in pure CoA-transferase. This suggests the presence of a minor contamination, by a proteinase in purified CoA-transferase samples. The study by White & Jencks (1976b) included inhibitors of serine proteinase. However, the possibility of other proteolytic enzymes being active during the purification cannot be excluded.

Molecular weights ranging from 78000 to 113000 have been reported for CoA-transferase isolated from various mammalian sources. Molecular weights of 102000 and 110000 have been obtained during the present studies for sheep kidney CoA-transferase by ultracentrifugation and chromatography on Sephadex G-200 respectively. These values are similar to that obtained by Edwards *et al.* (1973) for pig heart CoA-transferase (113000 obtained by chromatography on Sephadex G-200), and that of 100000 reported for various rat tissues (Fenselau & Wallis, 1974). A value of 92000 has also been reported for pig heart CoA-transferase (White & Jencks, 1976b).

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis indicates a subunit mol.wt. of 56000. Since only a single major band could be detected, in

samples stored at -20°C after chromatography on Blue Sepharose, the subunits of CoA-transferase have identical or very similar molecular weights. The presence of an additional two major bands in CoA-transferase preparations stored before chromatography on Blue Sepharose has been interpreted as indicating the presence of a discrete region in the protein that is susceptible to proteolytic cleavage.

It is interesting to compare these results with those obtained by Sramek & Frerman (1975) for the *E. coli* CoA-transferase. This bacterial enzyme is reported to exist as a tetramer with a $\alpha_2\beta_2$ arrangement of the subunits. The subunits have mol.wts. of 26000 (α) and 23000 (β). The resulting peptides from the cleavage of sheep kidney CoA-transferase have mol.wts. of 27000 and 24000 and are very similar to those values reported for the subunits of *E. coli* CoA-transferase.

No free *N*-terminal could be detected in sheep kidney CoA-transferase by the dansyl chloride method of *N*-terminal analysis, and peptide 'mapping' of tryptic digests of pure CoA-transferase has provided further evidence for sheep kidney CoA-transferase being a dimer of two identical subunits. The total number of arginine and lysine residues from the amino acid composition was found to be 110, based on a mol.wt. of 102000. If the subunits were different, the number of peptides from a tryptic digest would be expected to approach 112, the total number of arginine and lysine residues+2. If the subunits were

Table 4. Comparison of the amino acid composition of sheep kidney and pig heart CoA-transferase

Values for sheep kidney CoA-transferase have been calculated for a mol.wt. of 102000. The values for the pig heart CoA-transferase, reported for a mol.wt. of 92000 (White & Jencks, 1976b) have been adjusted for a mol.wt. of 102000 for comparison with the sheep kidney CoA-transferase.

	Sheep kidney	Pig heart
Lysine	73.4	60.6
Histidine	16.4	17.5
Arginine	35.2	33.2
Aspartate	81.5	83.5
Threonine	48	42.7
Serine	30	36
Glutamate	81	103.9
Proline	43	66.9
Glycine	93	91
Alanine	78	76.4
Cysteine	8	6.5
Valine	85.5	73.1
Methionine	23.4	4.4
Isoleucine	64	40.6
Leucine	85.5	74.2
Tyrosine	18.8	30.9
Phenylalanine	31.8	24.2
Tryptophan	3.4	19.7

identical, the expected number of peptides would approach 56, half the number of arginine and lysine residues+1. The number of peptides obtained after a tryptic digest was 43, suggesting that the composition and the sequence of the subunits is identical.

At present only the pig heart CoA-transferase (White & Jencks, 1976b) amino acid composition is available for comparison with that of sheep kidney CoA-transferase and the compositions show marked differences (Table 4). Sheep kidney CoA-transferase has a greater number of lysine residues and lower number of glutamic residues than the pig heart CoA-transferase, with 73.4 of the total number of residues being lysine residues and 81 being glutamic residues. These values are respectively 60.6 and 103.9 for the pig heart enzyme. Arginine and aspartic residues are similar for both proteins, being 35 and 82 respectively. This increase in the basicity of sheep kidney CoA-transferase is reflected in the high pI value (9) obtained for the enzyme, compared with pI of 5.5 obtained for the pig heart enzyme (pI 5.93 for the major peak obtained by White & Jencks, 1976b).

The amounts of proline, tryptophan and tyrosine residues are also decreased and the amounts of methionine, isoleucine and valine residues are increased in sheep kidney as compared with pig heart CoA-transferase. The values for sheep kidney CoA-transferase are 43, 3.4, 18.8, 23.4, 64 and 85.5 respectively, and for pig heart CoA-transferase they are 66.9, 19.7, 30.9, 24.4, 40.6 and 73.1 respectively.

The unusual pI of CoA-transferase from sheep kidney and the ease with which it can be purified has led to the preparation of homogeneous CoA-transferase with a specific activity of approx. 200 units/mg (pure pig heart CoA-transferase has a specific activity of 155 units/mg; Edwards *et al.*, 1973). This method of purification combined with the relatively high activity of CoA-transferase in sheep kidney makes feasible extensive studies into the structural properties of CoA-transferase. This method would also make CoA-transferase readily available for use in assay of acetoacetate and/or succinyl-CoA.

This work was supported by a grant from the Australian Research Grants Committee. J. A. S. is the recipient of a Deans Scholarship, awarded by the Faculty of Biological Sciences, University of New South Wales. We thank Ms. W. Fisher and Ms. P. Como for their helpful advice.

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