Biochem. J. (1976) **158**, 509–512
Printed in Great Britain

3-Oxo Acid Coenzyme A-Transferase in Normal and Diabetic Rat Muscle

By ALLAN FENSELAU and KATHLEEN WALLIS
The Johns Hopkins University School of Medicine, Department of Physiological Chemistry, Baltimore, MD 21205, *U.S.A.*

(*Received* 2 *June* 1976)

The amounts of succinyl-CoA–3-oxo acid CoA-transferase (EC 2.8.3.5) decrease progressively in skeletal muscle in streptozotocin-diabetic rats, reaching after 10 days about 50% of the value in normal rat muscle. Electrofocusing studies indicate the occurrence of partial proteolysis of the enzyme in diabetic muscle. However, several functional parameters relating to acetoacetate utilization, including substrate inhibition, are quite similar for muscle transferase preparations from normal and diseased rats. The development of pathological ketoacidosis is discussed in the light of these observations.

Previous studies in our laboratory (Fenselau & Wallis, 1974*a*) revealed the existence of substrate inhibition by acetoacetate of succinyl-CoA*–3-oxo acid CoA-transferase (EC 2.8.3.5), an initiator of ketone-body utilization in extrahepatic tissue. This effect, observed with enzyme preparations from heart, kidney, brain and skeletal muscle from normal adult rats, has not been studied for CoA-transferases from tissues of diabetic animals, where such an inhibition could contribute to the apparently impaired tissue utilization of ketone bodies in these animals and to their development of pathological ketosis (Beatty *et al.*, 1960; Balasse & Havel, 1971; Bates, 1972; Bässler *et al.*, 1973; Ruderman & Goodman, 1974). We have focused our studies on skeletal-muscle CoA-transferase in diabetic rats, because skeletal muscle is the major consuming tissue for ketone bodies (Bässler *et al.*, 1973) and because uncertainty exists about the extent of transferase activity in chronically diabetic rat muscle (Bässler *et al.*, 1973; Williamson *et al.*, 1971).

Experimental

Animals

Adult female Sprague–Dawley rats (150–200g) were obtained from Sprague–Dawley (Madison, WI, U.S.A.) and fed *ad libitum*. Starved animals were allowed only water for 48h before being killed. Diabetic ketoacidosis was produced by an intravenous injection of streptozotocin (90mg/kg; from Calbiochem, La Jolla, CA, U.S.A.). Diabetic rats were allowed free access to food and water and were used only if acutely ketotic as determined by an immediate strongly positive urine test for ketone bodies (Ketostix; Ames Co., Elkhart, IN, U.S.A.). Plasma concentrations of total ketone bodies in normal, starved and diabetic (3 days or more after streptozotocin treatment) rats were found to be 0.16 ± 0.05, 1.02 ± 0.08 and 4.85 ± 0.77mM respectively, by using a modification of the procedure of Williamson *et al.* (1962).

Preparation of tissue extracts and mitochondrial isolation

The procedures described previously (Fenselau & Wallis, 1974*b*; Fenselau *et al.*, 1975) were followed in order to obtain a total homogenate of hindquarter skeletal muscle in either 0.02M-potassium phosphate (pH7.0) or a Tris/KCl solution (pH7.4) containing 50mM-Tris/HCl, 0.1M-KCl, 5mM-$MgSO_4$, 1mM-ATP and 1mM-EDTA. The CoA-transferase activity in the phosphate medium, when necessary, could be concentrated by batchwise adsorption of the enzyme on alumina C_γ gel (Bio-Rad Laboratories, Richmond, CA, U.S.A.), followed by elution with a smaller volume of 0.25M-potassium phosphate (pH8.0). Protein concentrations were determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Muscle mitochondria were isolated by the procedure of Max *et al.* (1972) in a medium containing 10mM-Tris/HCl (pH7.4), 70mM-sucrose, 0.21M-mannitol and 0.1mM-EDTA. Uptake of O_2 in the presence and absence of acetoacetate was measured as previously described (Fenselau & Wallis, 1974*b*).

Determination of enzyme kinetic and molecular properties

Spectrophotometric measurements of CoA-transferase activity were made by detecting the formation of the Mg^{2+}–acetoacetyl-CoA complex by using the conditions described in detail elsewhere (Fenselau & Wallis, 1974*b*,*c*). Crude CoA-transferase in the total

* Succinyl-CoA is strictly 3-carboxypropionyl-CoA.

muscle homogenate was electrofocused on a 110ml-capacity isoelectric-focusing apparatus (LKB, Rockville, MD, U.S.A.), by using procedures described previously (Fenselau & Wallis, 1974*b*; Fenselau *et al.*, 1975).

Chemicals

All purchased chemicals were the highest purity available. β-Hydroxybutyrate dehydrogenase (EC 1.1.1.30) was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Results

CoA-transferase activities in the total homogenates of skeletal muscle from normal, 48h-starved, short-term (2–4 days) diabetic and long-term (over 4 days) diabetic rats were 6.84±0.44, 7.02±0.58, 4.96±0.82 and 3.19±0.31 μmol of acetoacetate formed/min per g wet wt. of tissue respectively. These values are averages (±S.E.M.) of duplicate measurements on four to six different preparations. No differences were found in the amounts of enzyme activity in muscle from normal and starved rats. However, in the diabetic animals' muscle, CoA-transferase activity decreased progressively with time after the streptozotocin injection, reaching after 10 days a value of less than half of the specific activity measured in the skeletal muscle of the normal animal.

The use of different homogenization media (potassium phosphate or Tris/KCl solutions) did not alter the results. These media were selected because CoA-transferase activity is generally more stable in a phosphate buffer (Fenselau & Wallis, 1974*b*) and the previously reported decrease in muscle transferase activity in diabetic rats was determined in a Tris/KCl buffer (Bässler *et al.*, 1973). Also, the presence of a mixture of various proteinase inhibitors [10mM-toluenesulphonyl fluoride, 2.8mM-1-chloro-4-phenyl-3-L-tosylamidobutan-2-one (TPCK) or -7-amino-1-chloro-3-L-tosylamidoheptan-2-one (TLCK)] in the homogenization medium did not lead to any increase in total transferase activity. No further decrease in activity was noted for solutions containing muscle CoA-transferase from normal or diabetic animals that were kept at 4°C over 6h. Thus transferase activity in skeletal muscle did decrease during the course of diabetes (in rats where no effort is made to maintain the animal on insulin) and this decrease was not due to proteolysis occurring as an artifact in the homogenization medium after cell lysis.

Several kinetic properties of muscle transferases from normal and long-term-diabetic rats were determined in order to compare these tissues' abilities to consume acetoacetate. The enzymes from both sources manifest the expected Ping Pong kinetic patterns and product inhibition by succinate that is competitive with respect to acetoacetate (Hersh & Jencks, 1967; Fenselau & Wallis, 1974*b*,*c*). Crude muscle transferase preparations, identically isolated from normal and diabetic rats, display similar K_m values for acetoacetate (0.58±0.04 and 0.60±0.05mM respectively) and K_i values for product inhibition by succinate (0.74±0.07 and 0.64±0.03mM respectively) (results are means±S.E.M. for duplicate measurements on two different muscle preparations). Substrate inhibition by acetoacetate (Fenselau & Wallis, 1974*a*) was also shown by both enzyme preparations, each revealing an inversion concentration of about 5mM-acetoacetate (corresponding to the concentration at the point of intersection for the two lines in Fig. 1). Similar effects were also observed with intact mitochondria from normal and diabetic rat skeletal muscle, the major difference being that in both cases inversion concentrations were significantly lower with mitochondria (about 0.75mM-acetoacetate). Thus it appears that the enzyme-kinetic effect of substrate inhibition is manifested by intact mitochondria in acetoacetate oxidation.

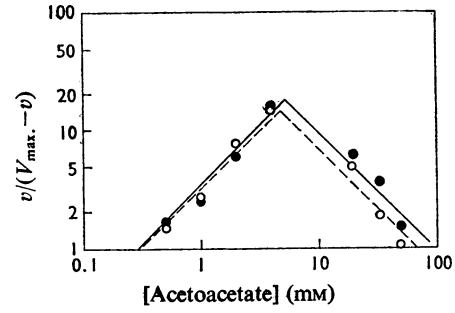

Fig. 1. *Acetoacetate substrate inhibition of CoA-transferase from normal and diabetic rat muscle*

Crude preparations of the muscle CoA-transferases from normal (○) and long-term-diabetic (●) rats were assayed at various concentrations of acetoacetate and 0.05mM-succinyl-CoA. The results are displayed in a Hill plot, obtained by determining $V_{max.}$ under these conditions from the double-reciprocal plot of the data and then calculating $v/V_{max.}$ at each experimental point.

Only one physical property of the transferases, namely the isoelectric point(s) of the active species, was examined in detail for the muscle transferases. Isoelectric focusing of the crude transferase preparations revealed distinctly different patterns for the enzymes from normal and long-term-diabetic rat skeletal muscle. The predominant active form (88%) of the enzyme from normal animals has pI 7.4; a lesser species is found with pI 6.8. For CoA-transferase preparations from diabetic animals, the active species were detected with pI values of 7.4 (69%), 6.8 (23%) and 6.3 (8%). Limited proteolysis of CoA-transferase from normal rat muscle by chymotrypsin or

trypsin decreased transferase activity to respectively 73 and 76% of the initial activity and shifted the pI value of the major active species in each of the treated preparations to 6.9 and 6.6 respectively. These observations suggest that the minor active species in the preparation from diabetic skeletal muscle are products of limited degradation of the enzyme and not different isoenzymic forms.

Discussion

The skeletal muscle of diabetic rats untreated with insulin, compared with normal tissue, contained less CoA-transferase activity after only several days, confirming the observation by Bässler *et al.* (1973). This decrease in activity is accompanied with a slight change in CoA-transferase protein composition detected by electrofocusing: the new active components of the mixture most probably arise from proteolysis within the cell during the diabetic period. The loss of enzyme activity therefore appears to be only one specific example of the general degradation of muscle tissue that is occurring in these animals.

This CoA-transferase mixture from the diabetic animals, two-thirds of which was the same in electrofocusing experiments as the major species obtained from muscle of normal rats, displayed the same kinetic properties as the enzyme isolated from normal rat muscle. That is, the values of the K_m for acetoacetate, K_i for succinate as a competitive product inhibitor and inversion concentration for acetoacetate as a substrate inhibitor are quite similar for enzyme preparations from normal and diabetic skeletal muscle. Substrate inhibition by acetoacetate was shown not only by preparations of muscle CoA-transferase but also by isolated muscle mitochondria; however, the inversion concentration for intact mitochondria, i.e. the concentration of acetoacetate where inhibition becomes evident in the Hill plot, is one-seventh of that in the enzyme preparations. A similar effect has been noted with mitochondria from rat kidney (Fenselau & Wallis, 1974*a*) and rat heart (A. Fenselau & K. Wallis, unpublished work). The lower inversion concentration for intact mitochondria may well be explained by the ability of mitochondria to transport and accumulate internally anions by a process that requires a pH difference across the mitochondrial membrane and possibly the existence of specific carriers (Klingenberg, 1970). It has been demonstrated that β-hydroxybutyrate and acetoacetate are transported by the carrier involved in pyruvate translocation (Mowbray, 1975; Paradies & Papa, 1975). In any event the occurrence of this or a similar concentrating effect permits this kinetic feature of CoA-transferase, namely substrate inhibition, to be operable under ketotic conditions in the rat and to play a role in controlling ketone-body metabolism.

Regulation of ketone-body metabolism in starved and diabetic rats, particularly with regard to the role of CoA-transferase, may be summarized as follows. In starvation the regulated fall in blood concentrations of insulin leads to increases in liver ketogenesis and in blood concentrations of ketone bodies. The plasma concentrations of ketone bodies reach a plateau, owing in part to the operation of the substrate inhibition by acetoacetate of CoA-transferase. This enzyme effect requires a diminished peripheral utilization of ketone bodies and their accumulation in the blood beginning at an approximate total concentration of 1 mM. Although several mechanisms by which ketone bodies feedback-inhibit their own production may normally be operable (e.g. effects on liver redox state and anti-lipolytic effects on adipose tissue) (for review, see Newsholme & Start, 1973), in diabetic animals the physiological interactions between ketone bodies and insulin become most significant. Ketone bodies, particularly acetoacetate, on the basis of studies in rats (Hawkins *et al.*, 1971; Malaisse & Malaisse-Lagae, 1968) and dogs (Madison *et al.*, 1964), can increase plasma insulin concentration, thereby decreasing fatty acid mobilization from adipose tissue and inhibiting ketogenesis. A deficiency in this process arises in diabetic rats, owing to the destruction of pancreatic β-cell function by selective streptozotocin treatment (Junod *et al.*, 1969). With the loss of insulin inhibition of ketogenesis, blood concentrations of ketone bodies continue to rise (past the plateau observed under starvation conditions) until the limits of renal clearance are reached.

In summary, the efficiency of extrahepatic tissues in utilizing ketone bodies is decreased in the short-term-diabetic rat compared with the starved rat, because blood concentrations of ketone bodies have attained values high enough for the display of a severe acetoacetate substrate-inhibitory effect on the CoA-transferase in these tissues. For the long-term-diabetic rat this grave situation is further exacerbated by decreases in muscle mass and muscle CoA-transferase content, with a consequent further decrease in ketone-body consumption.

We acknowledge helpful discussions with Dr. T. Spencer and the skilful typing of Mrs. Shirley Metzger. This study was supported by research grants from the National Science Foundation (GM-40233) and the U.S. Public Health Service, National Cancer Institute (CA-18053). A. F. is a recipient of a Research Career Development Award (GM-70423) from the National Institute of General Medical Sciences.

References

Balasse, E. O. & Havel, R. J. (1971) *J. Clin. Invest.* **50**, 801–813

Bässler, K.-H., Ackermann, R. H., Wagner, K. & Schonerstedt, B. (1973) *Hoppe-Seyler's Z. Physiol. Chem.* **354**,

Bates, M. W. (1972) *Am. J. Physiol.* **222**, 462–467
Beatty, C. H., Marco, A., Peterson, R. D., Bocek, R. M. & West, E. S. (1960) *J. Biol. Chem.* **235**, 2774–2777
Fenselau, A. & Wallis, K. (1974*a*) *Life Sci.* **15**, 811–818
Fenselau, A. & Wallis, K. (1974*b*) *Biochem. J.* **142**, 619–627
Fenselau, A. & Wallis, K. (1974*c*) *Biochemistry* **13**, 3884–3888
Fenselau, A., Wallis, K. & Morris, H. P. (1975) *Cancer Res.* **35**, 2315–2320
Hawkins, R. A., Alberti, K. G. M. M., Houghton, C. R. S., Williamson, D. H. & Krebs, H. A. (1971) *Biochem. J.* **125**, 541–544
Hersh, L. B. & Jencks, W. P. (1967) *J. Biol. Chem.* **242**, 3468–3480
Junod, A., Lambert, A. E., Stauffacher, W. & Renold, A. E. (1969) *J. Clin. Invest.* **48**, 2129–2139
Klingenberg, M. (1970) *Essays Biochem.* **6**, 119–159
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
Madison, L. L., Mebane, D., Unger, R. H. & Lochner, A. (1964) *J. Clin. Invest.* **43**, 408–415
Malaisse, W. J. & Malaisse-Lagae, F. (1968) *J. Lab. Clin. Med.* **72**, 438–448
Max, S. R., Garbus, J. & Wehman, H. J. (1972) *Anal. Biochem.* **46**, 576–584
Mowbray, J. (1975) *Biochem. J.* **148**, 41–47
Newsholme, E. A. & Start, C. (1973) in *Regulation in Metabolism*, Wiley–Interscience, New York, pp. 316–323
Paradies, G. & Papa, S. (1975) *FEBS Lett.* **52**, 149–152
Ruderman, N. B. & Goodman, M. N. (1974) *Am. J. Physiol.* **226**, 136–143
Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962) *Biochem. J.* **82**, 90–96
Williamson, D. H., Bates, M. W., Page, M. A. & Krebs, H. A. (1971) *Biochem. J.* **121**, 41–47