Biochimica et Biophysica Acta, 309 (1953) 457–465

BBA 66903

LIVER MALONYL-CoA DECARBOXYLASE

H. R. SCHOLTE*

*Laboratory of Biochemistry, B.C.P. Jansen Institute**, University of Amsterdam, Amsterdam (The Netherlands)*

(Received December 18th, 1972)

SUMMARY

1. Malonyl-CoA decarboxylase (EC 4.1.1.9) was purified 51-fold from beef liver homogenate, by acid extraction, ammonium sulphate fractionation, precipitation at pH 5.8 and gel chromatography on Sephadex G-200. The molecular weight was estimated to be 250 000. Phosphate is an activator of the enzyme. The velocity measured at a high substrate concentration (several times K_m) is constant between 40 and 200 mM phosphate. Within this range, the K_m for malonyl-CoA decreases with increasing phosphate concentrations. The enzyme possesses optimal activity at pH 5 and 9, and a minimal activity between pH 6 and 7.

2. Acetyl-CoA, propionyl-CoA and palmitoyl-CoA are non-competitive inhibitors with K_i values of 0.66 mM, 0.28 mM and 2.3 μM, respectively. The inhibitory action of palmitoyl-CoA decreases with increasing protein concentrations. The enzyme is partially competitively inhibited by aromatic aldehydes.

3. The malonyl-CoA decarboxylase activity of intact mitochondria is latent, and this latency is not abolished by the addition of carnitine. Malonyl-CoA is not a substrate for carnitine acetyltransferase (EC 2.3.1.7). Since malonyl-CoA decarboxylase is located in the mitochondrial matrix space, it cannot affect the extramitochondrial malonyl-CoA.

INTRODUCTION

With the aid of marker enzymes malonyl-CoA decarboxylase (EC 4.1.1.9) has been localized in the mitochondria in rat liver[1] and guinea pig heart[2], and within mitochondria in the matrix space[1–6]. In both rat liver[1,7] and guinea pig heart[4,5] mitochondria, malonyl-CoA decarboxylase is relatively loosely bound to the inner side of the inner mitochondrial membrane. It is less loosely bound than malate dehydrogenase (EC 1.1.1.37), about equally strong as glutamate dehydrogenase (EC 1.4.1.3), and more strongly bound than propionyl-CoA carboxylase (EC 6.4.1.3).

* Present address: Department of Biochemistry I, Rotterdam Medical School, Erasmus University Rotterdam, P.O. Box 1738, Rotterdam, The Netherlands.

** Postal address: Plantage Muidergracht 12, Amsterdam, The Netherlands.

In the present paper a procedure is given for the isolation of liver malonyl-CoA decarboxylase and some properties of the isolated enzyme are described, including inhibition by other coenzyme A esters. The purified enzyme was used as a tool to establish the possibility of malonyl transfer through the mitochondrial membrane.

METHODS

The methods used are given in ref. 1. Malonyl-CoA decarboxylase was assayed with 0.62 mM [1(3)-^{14}C]malonyl-CoA, in a volume of 0.5 ml at 25 °C. The rate of the reaction was constant and was proportional to the enzyme concentration until more than 30% of the malonyl-CoA had been decarboxylated. The enzyme activity is given in μmoles malonyl-CoA decarboxylated per min (units).

RESULTS

Isolation

About 1 kg of liver was transferred, as soon as possible after the death of the cow, into ice-cold 0.25 M sucrose containing 0.002% chlorhexidine. Fat and connective tissue, including the fascia hepatica were removed in a cold room. The liver was sliced and the blood vessels removed as far as possible. Portions of 150 g of liver were washed with 100 ml medium, and homogenized in a final volume of 1 l. Homogenization was carried out in a Braun mixer for successive 30-s periods at speed positions 1, 2 and 3, (12 000, 15 000 and 20 000 rev./min), respectively. The further isolation was carried out at 0 °C. The combined homogenates were centrifugated for 10 min at 900 $\times$ g_{max}. The supernatant was collected by siphoning, and after filtering through two layers of cheese cloth, it was continuously centrifugated for 2.5 h at 30 000 $\times$ g_{max}. The sediment, the mitochondrial fraction, was taken up in 0.25 M sucrose containing 5 mM 2-mercaptoethanol. Unless otherwise stated 5 mM 2-mercaptoethanol was present in all the solutions used in the isolation.

The mitochondrial fraction was briefly homogenized in the mixer, and under stirring the suspension was acidified to pH 4.4 by the dropwise addition of 0.25 M acetic acid. After 1 h stirring the suspension was adjusted to pH 7.0 with powdered K_2HPO_4, and centrifugated for 1 h at 65 000 $\times$ g_{max}. The yellow supernatant was dialysed overnight against 25 l of 20 mM potassium phosphate buffer (pH 7.0). The dialysate was fractionated with saturated (0 °C), neutral, ammonium sulphate, the fraction precipitating between 30 and 50% saturation being collected by centrifugation for 20 min at 5000 $\times$ g_{max}, dissolving in 5 mM 2-mercaptoethanol, and dialysing overnight against 6 l 20 mM potassium phosphate buffer (pH 7.0). The enzyme was precipitated from the dialysed solution by the dropwise addition under stirring of 0.25 M acetic acid untila, pH 5.8 was reached. This purification step is only successful at a low ionic strength and a high protein concentration. The sediment collected by centrifugation for 5 min at 30 000 $\times$ g_{max} was taken up in 0.15 M potassium phosphate buffer (pH 7.0) and chromatographed on Sephadex G-200 (93 cm long and 2.1 cm diameter), using as eluant 0.15 M potassium phosphate buffer (pH 7.0) to which 6 drops of toluene were added per l as a bacteriostatic agent. The flow rate was 0.2 ml/min. The most active fractions of 4 ml each were collected and centrifuged for 5 min at 30 000 $\times$ g_{max}. The supernatant was again subjected to

TABLE I

THE ISOLATION OF MALONYL-CoA DECARBOXYLASE FROM BEEF LIVER

Stage	*Volume (ml)*	*Total activity (units)*	*Protein (g)*	*Specific activity (units/g)*
1 Homogenate	6000	678	168	4.03
2 Nuclear-free homogenate	4800	452	120	3.78
3 Mitochondrial fraction	330	245	35.2	6.97
4 After acidification and dialysis	505	194	11.7	16.6
5 30–50% $(NH_4)_2SO_4$ sediment	55.5	184	4.77	38.6
6 After dialysis	79.0	138	4.54	30.4
7 pH 5.8 precipitate	10.5	93.6	1.19	78.8
8 Sephadex G-200	81.5	88.4	0.707	125
9 30–50% $(NH_4)_2SO_4$ sediment	14.1	87.3	0.424	206

ammonium sulphate fractionation. This preparation, solubilized in a minimal volume of 5 mM 2-mercaptoethanol, was stored in a refrigerator. A typical example of a purification protocol is given in Table I.

The most active preparation obtained during various attempts at purifying the enzyme had a specific activity of 690 units/g protein, but only a very low yield of enzyme of this purity was obtained. The procedure described gives a much higher yield of a product of reasonable purity. After 4 months at −20 °C one-third of the enzyme activity had disappeared.

The estimation of the molecular weight

The molecular weight was estimated by gel chromatography on Sephadex G-200, with 0.15 M phosphate buffer (pH 7.0), 5 mM 2-mercaptoethanol and 0.002% chlorhexidine as elution fluid. At this ionic strength the enzyme stays completely

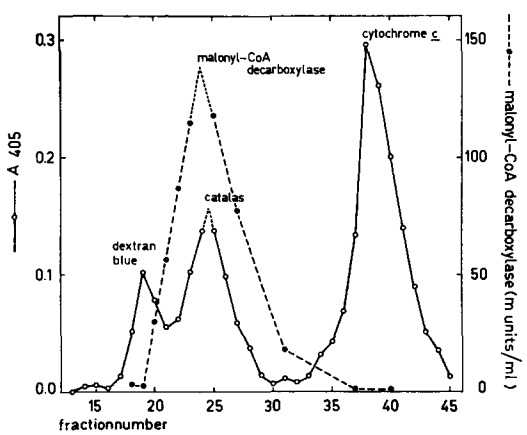


Fig. 1. Gel chromatography of malonyl-CoA decarboxylase. A column with Sephadex G-200, long 75.1 cm, diameter 2.03 cm, bed volume 244 ml, was calibrated with 1.95 mg dextran blue, 1.69 mg catalase and 1.35 mg cytochrome *c* in 2.5 ml 0.4 M sucrose. 200 mg malonyl-CoA decarboxylase (6.6 units) of the first ammonium sulphate precipitate (see Table I) dissolved in 2.5 ml 5 mM 2-mercaptoethanol–0.4 M sucrose was applied to the column. The elution fluid was 0.15 M phosphate buffer (pH 7.0), 5 mM 2-mercaptoethanol and 0.002% hibitane. The flow rate was 0.34 ml/min. Fractions of 6.88 ml were collected. The temperature was 5–7 °C.

in solution. The column was calibrated with dextran blue (mol. wt $> 2 \cdot 10^6$), catalase (EC 1.11.1.6) from beef liver (mol. wt 225 000), and cytochrome *c* from beef heart (mol. wt 13 000). Malonyl-CoA decarboxylase eluted just before catalase (Fig. 1). The molecular weight of malonyl-CoA decarboxylase in 0.15 M phosphate buffer is estimated to be 250 000, with a possible error of 10% (Fig. 2).

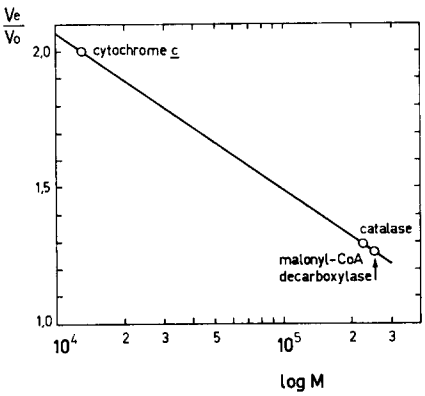


Fig. 2. Estimation of the molecular weight of malonyl-CoA decarboxylase. The elution volumes of Fig. 1 (V_e), divided by the elution volume of the dextran blue (V_0) are plotted as a function of log mol. wt If malonyl-CoA decarboxylase is a globular protein (like most enzymes) the molecular weight can be estimated to be 250 000.

When the chromatography is carried out with 40 mM phosphate buffer of pH 7.0 as elution fluid, the enzyme precipitates and elutes partly together with dextran blue.

The effect of phosphate on the enzyme activity

Potassium phosphate buffer (pH 7.0) stimulates the enzyme activity (Fig. 3). Between 40 and 200 mM phosphate the reaction velocity, measured at a high con-

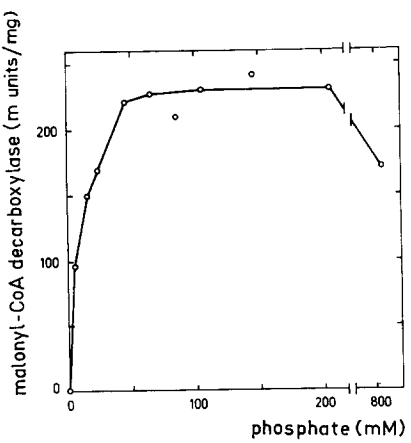


Fig. 3. The effect of phosphate on the activity of isolated malonyl-CoA decarboxylase (42 μg/ml). Potassium phosphate buffer (pH 7.0) was used.

centration of malonyl-CoA (0.62 mM), is practically constant, but increasing the phosphate concentration within this range lowers considerably the K_m of both rat and beef liver enzyme (Table II). Since the potassium phosphate could not be replaced by KCl as activator, it is likely that the anion is responsible.

TABLE II

THE EFFECT OF PHOSPHATE ON THE K_m FOR MALONYL-CoA

The supernatant of digitonin-treated rat liver mitochondria was prepared as described in ref. 1. Phosphate was added as potassium phosphate buffer (pH 7.0). Different enzyme preparations were used in the last two lines. In the 4th line the second ammonium sulphate sediment (Stage 9, Table I) was used, and in Line 5 the first one (Stage 5, Table I).

Preparation	*Phosphate (mM)*	K_m *(mM)*
Supernatant of digitonin-treated rat liver mitochondria	40	0.127
	100	0.093
	200	0.047
Purified enzyme from beef liver mitochondria	40	0.146
	150	0.065

The effect of pH and temperature

The malonyl-CoA decarboxylase reaction is always minimal between pH 6 and 7 (Fig. 4). Independent of the ionic strength of the medium, and independent of the purity of the enzyme preparation, this reaction is optimal at about pH 5 and 9. The velocities of the malonyl-CoA decarboxylase reaction catalysed by the first ammonium sulphate sediment (see Table I) in the presence of 20 mM KCl and a mixture of 66 mM citric acid and 0.2 M Tris (in order to obtain the desired pH value) were for pH 4.5, pH 5.0, pH 9.0 and pH 9.5: 0.92, 1.26, 1.23 and 0.93 times the velocity measured at pH 6.5, respectively. The possibility exists that malonyl-CoA is decarboxylated by the same enzyme by different reaction mechanisms in acidic and alkaline solutions, but the existence of isoenzymes cannot completely be excluded.

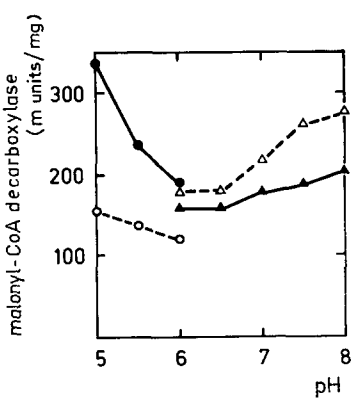


Fig. 4. The effect of the pH on the activity of isolated malonyl-CoA decarboxylase. ○ and ●, potassium acetate buffers; △ and ▲, potassium phosphate buffers. ○ and △, 42 μg enzyme/ml, I 0.11; ● and ▲, 74 μg enzyme/ml, I 0.28.

The behaviour of beef liver malonyl-CoA decarboxylase is in contrast to that reported for the yeast enzyme, with an optimum at pH 6.4 (ref. 8) and for the enzyme in disrupted rat liver mitochondria with an optimum at pH 7 (ref. 6). It is possible that the discrepancies can be explained by the malonyl-CoA concentrations used, which in the present experiments were much higher than the K_m, whereas concentrations of malonyl-CoA equal to or lower than the K_m were used in previous reports.

The Q_{10} of the reaction, catalysed by 10 μg purified enzyme and measured between 5 and 37 °C (7 points) was 1.78. Half of the enzyme is inactivated by pre-incubation for 2 min at 49 °C, before the assay of the enzyme at 25 °C. The Q_{10} of the inactivation is 37.

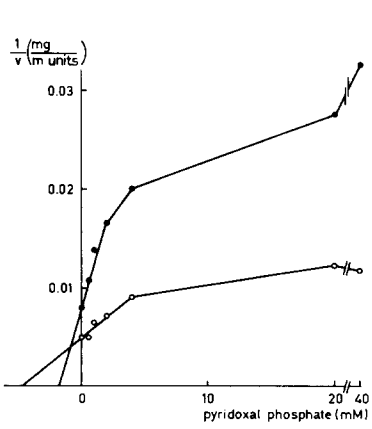
Inhibitors of malonyl-CoA decarboxylase

No evidence was obtained that the enzyme requires Mg^{2+} for activity. EDTA (6 mM) had no effect and Mg^{2+} (50 mM) inhibited by 46%, probably by interaction with the activator phosphate (150 mM present). The lack of requirement of Mg^{2+} is in agreement with the report of Hayaishi[9] with respect to the bacterial enzyme, but in disagreement with that of Wolfe *et al.*[10], dealing also with the bacterial enzyme.

The activity is inhibited by aromatic aldehydes. Pyridoxal 5'-phosphate is a partly competitive inhibitor (Fig. 5). Vanillin behaves similarly. Obviously the enzyme is only partially inhibited when $-NH_2$ groups of the enzyme are converted into Schiff bases.

Other coenzyme A esters are non-competitive inhibitors (*e.g.* Fig. 6). Acetyl-CoA and propionyl-CoA inhibit with K_i values of 0.66 and 0.28 mM, respectively. These inhibitions are not affected by the protein concentration used. Malonate (20 mM) and $KHCO_3$ (20 mM) are not inhibitory.

Palmitoyl-CoA also inhibits the enzyme non-competitively with a K_i value of 2.3 μM at a protein concentration of 44.6 μg/ml. It is possible that the palmitoyl-CoA inhibition correlates with its critical micellar concentration, and that the inhi-



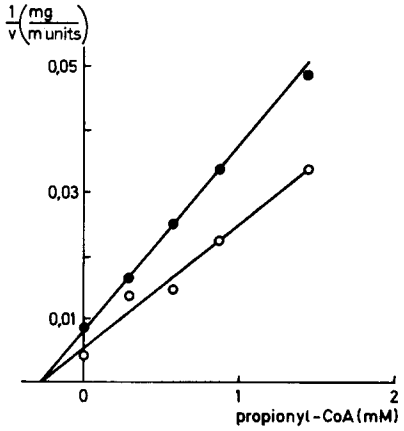


Fig. 5. Inhibition of isolated malonyl-CoA decarboxylase by pyridoxal 5'-phosphate. ○, 0.62 mM malonyl-CoA; ●, 0.123 mM malonyl-CoA. 44.6 μg/ml enzyme were used.

Fig. 6. Inhibition of isolated malonyl-CoA decarboxylase by propionyl-CoA. ○, 0.62 mM malonyl-CoA; ●, 0.123 mM malonyl-CoA. 44.6 μg/ml enzyme were used. The K_i is equal to 0.28 mM.

bition is due to the surfactant property of the ester[11]. L-Palmitoylcarnitine is less inhibitory. 50 μM inhibits 90% at a protein concentration of 36 μg/ml. The addition of 3.6 μM palmitoyl-CoA or 50 mM L-palmitoylcarnitine has no effect on the enzymic activity of 0.5 mg sonicated rat liver mitochondria. Landriscina *et al.*[6] found with 0.3 mg sonicated rat liver mitochondria uncompetitive inhibition by 100 μM palmitoyl-CoA.

[3-^{14}C]Methylmalonyl-CoA, the product of the propionyl-CoA carboxylase reaction in the presence of $KH^{14}CO_3$, is not decarboxylated by the purified enzyme.

Carnitine and the metabolism of malonyl-CoA in mitochondria

The addition of DL-carnitine stimulates the malonyl-CoA decarboxylase activity of mitochondria somewhat, but the activity is still low compared with that of Lubrol-treated mitochondria (Table III).

Table IV (Line 1) shows that carnitine acetyltransferase does not catalyse the transfer of the acyl group of malonyl-CoA to carnitine. Only after addition of malonyl-

TABLE III

THE EFFECT OF CARNITINE ON THE MALONYL-CoA DECARBOXYLASE ACTIVITY OF INTACT RAT LIVER MITOCHONDRIA

The reaction was measured in 0.25 M sucrose buffered with 4 mM potassium phosphate buffer (pH 7.0).

DL-*Carnitine (mM)*	*Lubrol (μg)*	*Malonyl-CoA decarboxylase activity (munits/mg)*
0	0	0.62
2	0	0.94
8	0	1.11
32	0	1.50
136	0	1.16
0	50	9.19

TABLE IV

THE FORMATION OF ACETYLCARNITINE FROM ACETYL-CoA FORMED BY DECARBOXYLATION OF MALONYL-CoA

The reaction mixtures (2.15 ml) contained 55 mM potassium phosphate buffer (pH 7.0), 0.144 mM [1(3)-^{14}C]malonyl-CoA (27.6 nCi) and additions as indicated. The reaction was carried out simultaneously in Warburg vessels and in quartz cuvettes. In the cuvettes $A_{232\ nm}$ was measured. From the decline of $A_{232\ nm}$, the loss of acyl-CoA was calculated assuming that $A_{232\ nm}$ (acyl-CoA *minus* CoASH) is 4.5 $mM^{-1} \cdot cm^{-1}$ (ref. 17). The incubations were carried out for 5 min at 25 °C.

Additions			*Loss of acyl-CoA (nmoles)*	$^{14}CO_2$ *formed (nmoles)*
DL-*Carnitine (mM)*	*Carnitine acetyltransferase (units)*	*Malonyl-CoA decarboxylase (munits)*		
1.86	2	0	0.0	0.0
0	2	12	0.0	58.3
1.86	0	12	12.9	55.8
1.86	2	12	66.4	62.9

CoA decarboxylase together with carnitine (Lines 3 and 4) is there a loss of acyl-CoA. The formation of acetylcarnitine in the absence of added carnitine acetyltransferase (Line 3) indicates that the latter enzyme is present as an impurity in the malonyl-CoA decarboxylase preparation.

Bremer[12] has shown that carnitine acetyltransferase is also inactive with succinylcarnitine. It seems then that this enzyme cannot convert CoA esters of dibasic carboxylic acids into their respective carnitine esters.

DISCUSSION

Malonyl-CoA, an intermediate in extramitochondrial fatty acid synthesis taking place both in the cytosol and in the endoplasmatic reticulum, can be decarboxylated as such only within the mitochondrial inner membrane. The malonyl-CoA decarboxylase activity of purified acetyl-CoA carboxylase and the *de novo* fatty acid synthesizing complex (see refs 1, 13) is most probably due to contamination with malonyl-CoA decarboxylase, that has leaked out of the mitochondria. *In vitro*, however, a malonyl-CoA decarboxylase activity can originate from the *de novo* fatty acid synthesizing complex by treatment with iodoacetamide, as has been shown with the purified yeast enzyme[14].

Like other acyl-CoA compounds malonyl-CoA does not penetrate the mitochondrial inner membrane but, unlike the coenzyme A esters of the monobasic acids, it does not react with carnitine to form the lipid-soluble carnitine ester. Thus, extramitochondrial malonyl-CoA is protected from the action of mitochondrial malonyl-CoA decarboxylase.

It has been suggested elsewhere[1,2,15] that malonyl-CoA has no function in the mitochondria, that it is synthesized by a side reaction of propionyl-CoA carboxylase[16], and that the physiological function of malonyl-CoA decarboxylase is to decarboxylate the 'accidentally' formed malonyl-CoA, which would otherwise remove CoASH from the system.

ACKNOWLEDGEMENTS

The author wishes to thank Professor E. C. Slater for his stimulating advice and criticism, and Mrs Dr E. M. Wit-Peeters for many helpful discussions. The expert technical assistance of Mr H. L. A. Pijst is gratefully acknowledged. The present investigations have been carried out under the auspices of the Netherlands Foundation for Chemical Research (S.O.N.) and with financial aid from the Netherlands Organization for the Advancement of Pure Scientific Research (Z.W.O.).

REFERENCES

1 Scholte, H. R. (1969) *Biochim. Biophys. Acta* 178, 137–144
2 Scholte, H. R. (1970) *Het Metabolisme van Malonyl-CoA in Mitochondriën*, Ph. D. Thesis, University of Amsterdam, pp. 1–87, Mondeel Offsetdrukkerij, Amsterdam
3 Landriscina, C., Papa, S. Coratelli, P., Mazzarella, L. and Quagliariello, E. (1970) *Biochim. Biophys. Acta* 205, 136–147
4 Wit-Peeters, E. M., Scholte, H. R., van den Akker, F. and de Nie, I. (1971) *Biochim. Biophys. Acta* 231, 23–31

5 Scholte, H. R., Wit-Peeters, E. M. and Bakker, J. C. (1971) *Biochim. Biophys. Acta* 231, 479–486
6 Landriscina, C., Gnoni, G. V. and Quagliariello, E. (1971) *Eur. J. Biochem.* 19, 573–580
7 Wit-Peeters, E. M. (1969) *Biochim. Biophys. Acta* 176, 453–462
8 Lynen, F., Domagk, G. F., Goldmann, M. and Kessel, I. (1962) *Biochem. Z.* 335, 519–539
9 Hayaishi, O. (1953) *J. Am. Chem. Soc.* 75, 4367
10 Wolfe, J. B., Ivler, D. and Rittenberg, S. C. (1954) *J. Biol. Chem.* 209, 875–883
11 Barden, R. E. and Cleland, W. W. (1969) *J. Biol. Chem.* 244, 3677–3684
12 Bremer, J. (1968) in *Cellular Compartmentalization and Control of Fatty Acid Metabolism* (Gran, F. C., ed.), pp. 65–88, Universitetsforlaget, Oslo, and Academic Press, London and New York
13 Hansen, H. J. M., Carey, E. M. and Dils, R. (1971) *Biochim. Biophys. Acta* 248, 391–405
14 Sumper, M., Oesterhelt, D., Riepertinger, C. and Lynen, F. (1969) *Eur. J. Biochem.* 10, 377–387
15 Christ, E. J. V. J. (1968) *Biochim. Biophys. Acta* 152, 50–62
16 Hülsmann, W. C. (1966) *Biochim. Biophys. Acta* 125, 397–400
17 Stadtman, E. R. (1957) in *Methods in Enzymology* (Colowick, S. P. and Kaplan, N. O., eds), Vol. III, pp. 936–937, Academic Press, New York