

Effect of aging and acetyl-L-carnitine on the activity of cytochrome oxidase and adenine nucleotide translocase in rat heart mitochondria

Giuseppe Paradies*, Francesca Maria Ruggiero, Giuseppe Petrosillo, Maria Nicola Gadaleta, Ernesto Quagliariello

Department of Biochemistry and Molecular Biology and C.N.R. Unit for the Study of Mitochondria and Bioenergetics, University of Bari, Bari, Italy

Received 24 June 1994

Abstract

The effect of aging and treatment with acetyl-L-carnitine on the activity of cytochrome oxidase and adenine nucleotide translocase in rat heart mitochondria was studied. It was found that the activity of both these mitochondrial protein systems was reduced (by around 30%) in aged animals. Treatment of aged rats with acetyl-L-carnitine almost completely reversed this effect. Changes in the mitochondrial cardiolipin content appear to be responsible for these effects of acetyl-L-carnitine.

Key words: Cytochrome oxidase; ADP translocase; Aging; Acetyl-L-carnitine; Rat heart mitochondria

1. Introduction

Aging is associated with a decline in cardiac functional competence. The molecular basis of this biological phenomenon is still not well understood. The well recognized age-dependent decrement in heart performance may be related to age linked changes in the mitochondrial membranes lipids which influence the activity of diverse membrane bound enzymes and proteins (for review see [1]).

Acetyl-L-carnitine is a natural biomolecule which acts by stimulating energy metabolism [2–4] although its molecular mechanism of action is still not well known. However many different effects of acute treatment of aged rats with this compound have been reported [5–7].

We have recently reported that heart mitochondrial cardiolipin content is decreased with aging and that treatment of aged rats with acetyl-L-carnitine restores the normal level of this phospholipid in the inner mitochondrial membrane [8]. These changes in the cardiolipin content have been implicated in altered rates of the mitochondrial phosphate transport in aging. Cardiolipin is also required for optimal activity of cytochrome oxidase and adenine nucleotide translocase in mitochondria [9–13].

This study examined the effect of aging and acetyl-L-carnitine treatment on the activity of cytochrome oxidase and adenine nucleotide translocase in rat heart mitochondria.

2. Materials and methods

Male Fisher rats of 5 months (young) and 28 months (aged) were used for these studies. In each experiment, two rats of 5 months and two rats of 28 months were injected intraperitoneally with 300 mg/kg b.wt. of acetyl-L-carnitine [5] and killed three hours after.

Rat heart mitochondria were prepared by differential centrifugation of rat homogenates as described previously [14].

Mitochondrial cytochrome oxidase activity was measured polarographically, essentially as described in [15].

The ADP transport in mitochondria was measured by the stop inhibitor method, using carboxyatractyloside (CAT) essentially as described in [16].

The content of ADP translocator was determined by titrating the rate of ADP transport with increasing concentrations of CAT [17–18].

Protein concentration was measured by the usual biuret method using serum albumine as standard.

3. Results

The kinetic parameters of the cytochrome oxidase in heart mitochondria from young, young plus acetyl-L-carnitine, aged and aged plus acetyl-L-carnitine treated rats are reported in Table 1. While there was practically no change in the K_m values of cytochrome *c* for its oxidase among these four types of mitochondria, the maximal activity of this enzymatic system was markedly reduced (around 30%) in mitochondria from aged rats. Treatment of aged rats with acetyl-L-carnitine restored the activity of the cytochrome oxidase to the level of young rats. This compound had practically no effect on the activity of cytochrome oxidase in mitochondria isolated from young rats.

The activity of cytochrome oxidase is known to be dependent on the phospholipid composition of the mitochondrial membranes. To examine the possible role of changes in bulk membrane lipids in affecting oxidase function, the temperature dependence of the oxidase ac-

*Corresponding author. Dipartimento di Biochimica e Biologia Molecolare, Università degli Studi di Bari, via E. Orabona 4, 70126 Bari, Italy.

Abbreviations: Ac. Carn., Acetyl-L-carnitine; CAT, carboxyatractyloside; TMPD, *N,N,N',N'*-tetramethyl-p-phenylenediamine.

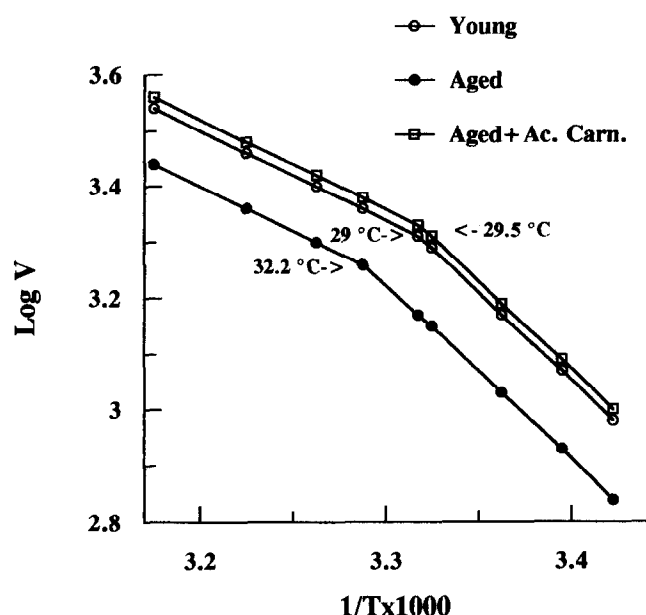


Fig. 1. Temperature dependence of cytochrome *c* oxidase in heart mitochondria from young, aged and aged + acetyl-L-carnitine treated rats. The activity of cytochrome oxidase in mitochondria was determined at various temperatures, essentially as described in the legend to Table 1. Cytochrome *c* was added at concentration of 20 μ M. Oxygen concentration of the medium at each temperature was calibrated by recording the stoichiometric oxidation of a limiting amount of spectrophotometrically standardized NADH in separate experiments. The experiment shown is representative of four experiments which gave similar results.

tivity in mitochondria isolated from young, aged and aged + acetyl-L-carnitine treated rats was examined. The Arrhenius plots of a typical experiment of cytochrome oxidase activity in mitochondria from these three types of mitochondria are reported in Fig. 1. Mitochondrial preparation from young rats exhibited a biphasic plot of log respiration rate versus reciprocal temperature, with change in the slope at $29.0 \pm 3.1^\circ\text{C}$. With heart mitochondria preparation from aged rats, the Arrhenius plot for cytochrome oxidase activity was also biphasic, but the position of the breakpoint was shifted to a higher temperature $32.2 \pm 3.4^\circ\text{C}$. This difference in temperature dependence of cytochrome oxidase activity in mitochondria from young and aged rats indicates that the lipid microenvironment of the active enzyme molecules in these two types of mitochondrial membranes is not the same. Treatment of aged rats with acetyl-L-carnitine restored the altered Arrhenius plot profile for mitochondrial cytochrome *c* oxidase to that observed in mitochondria from young rats.

It has been shown that the activity of the ADP translocase is decreased in heart mitochondria from aged rats [19–20]. This decreased activity was ascribed to an altered membrane lipid environment of this carrier protein. The effect of treatment of aged rats with acetyl-L-carnitine on the activity of the ADP carrier in heart

mitochondria was studied. The results reported in Table 2 show that the activity of the ADP translocase, measured as rate of ADP uptake by mitochondria, was reduced (by more than 30%) with aging. Pretreatment of aged rats with acetyl-L-carnitine restored the activity of this carrier system to the level of young rats. Acetyl-L-carnitine treatment had no effect on the ADP carrier activity in mitochondria from young animals.

In addition, the results reported in Table 2 show that the content of ADP carrier, obtained from the minimal concentration of CAT required to completely inhibit the translocase, was practically the same in heart mitochondria isolated from young, aged and aged + acetyl-L-carnitine treated rats. This indicates that the capability of acetyl-L-carnitine to bring back the reduced mitochondrial ADP carrier activity of aged rats to the level of young rats is not due to an increase in ADP carrier molecules.

4. Discussion

The results presented herein indicate that the activity of both cytochrome oxidase and adenine nucleotide translocase is reduced in heart mitochondria from aged rats. Treatment of aged rats with acetyl-L-carnitine almost completely restores the reduced activity of these two protein systems to the level of young control rats.

The activity of both cytochrome oxidase and adenine nucleotide translocase has been shown to be dependent upon the presence of cardiolipin, a phospholipid local-

Table 1

Kinetic parameters of the cytochrome oxidase in heart mitochondria from young, young + acetyl-L-carnitine, aged and aged + acetyl-L-carnitine treated rats

Animals	Cytochrome oxidase		Decrement
	K_m (μ M)	V_{max} (natom O/min/mg prot.)	
Young	10.5 ± 0.7	$3,220 \pm 350$	
Young + Ac. Carn.	10.9 ± 0.8	$3,280 \pm 340$	
Aged	11.3 ± 0.8	$2,280 \pm 290^*$	–29%
Aged + Ac. Carn.	11.1 ± 0.8	$3,190 \pm 340$	

Cytochrome oxidase activity in mitochondria was measured polarographically with an oxygen electrode at 25°C , in a medium containing 100 mM KCl, 20 mM Na-HEPES and 5 mM MgCl_2 (final pH 7.4). For K_m determinations 10 mM ascorbate, 0.05 mg Triton X-100, 1 mM TMPD, varying concentrations of cytochrome *c* (1–100 μ M), and 0.1–0.15 mg of mitochondrial protein were present in a final volume of 1 ml. Horseheart cytochrome *c* (type IV, Sigma, St. Louis, MO) was used for all experiments. Rates of oxygen uptake were corrected for autooxidation measured in the absence of mitochondria. Kinetic parameters were determined graphically from Lineweaver–Burk double-reciprocal plots. Each value represents the mean $\pm$ S.E. obtained for four separate experiments with two rats of each group.

* $P < 0.01$ vs. young rats.

Table 2

Rate of ADP transport and content of ADP carrier in heart mitochondria from young, young + acetyl-L-carnitine, aged and aged + acetyl-L-carnitine treated rats

Animals	ADP transport (nmol/min/mg protein)	Content of ADP carrier (nmol/mg protein)
Young	18.8 ± 1.4	1.12 ± 0.12
Young + Ac. Carn.	19.4 ± 1.7	1.10 ± 0.14
Aged	13.0 ± 1.3*	1.08 ± 0.15
Aged + Ac. Carn.	18.6 ± 1.5	1.10 ± 0.14

The rate of [14 C]ADP transport was measured at 0°C with a medium made of 120 mM KCl, 10 mM Tris-HCl, 1 mM EDTA (final pH 7.2; final volume 1 ml). Mitochondria (1 mg of protein) were preincubated in the reaction medium for 2 min prior to addition of radiolabelled ADP (100 μ M). The incubation lasted for 10 s and stopped by 20 μ M CAT. ADP transport was considered as difference of uninhibited and CAT-treated samples. The content of ADP translocator was determined by titrating the rate of ADP transport with increasing concentrations of CAT. Experimental conditions are similar to those described above with concentrations of CAT going from 0.1 to 2 μ M. The content of the active adenine nucleotide translocator was determined by the minimal amount of CAT sufficient to completely inhibit the ADP transport. Since the binding of CAT to the translocase is essentially stoichiometric, the minimal concentration of this inhibitor required to completely inhibit the translocase, can be taken as quantitative expression of the translocase content. Each value represents the mean $\pm$ S.E. obtained for four separate experiments with two rats of each group.

* $P < 0.01$ vs. young.

ized at the level of the inner membrane of mitochondria where it is biosynthesized [9–13]. In a previous paper we have shown that the cardiolipin content is reduced by more than 30% in mitochondria from aged rats as compared to young rats [8]. This reduced level of cardiolipin is brought back to the level of young rats by treatment of aged rats with acetyl-L-carnitine. These changes in cardiolipin content are associated with parallel changes in the activity of both cytochrome oxidase and ADP translocase (present results). Thus, the most obvious explanation of the effect of acetyl-L-carnitine on the activity of both cytochrome oxidase and ADP translocase in mitochondria from aged rats is the restoration of the normal level of cardiolipin which is required for optimal activity of these two protein systems in mitochondria.

The molecular mechanism remains to be ascertained by which acetyl-L-carnitine restores the normal level of cardiolipin in cardiac mitochondrial membrane from aged rats. One possibility is that acetyl-L-carnitine may affect the activity of mitochondrial cardiolipin synthase,

the enzyme protein involved in the cardiolipin biosynthesis, as recently suggested for the effect of thyroxine on the activity of this enzyme in rat liver mitochondria [21].

Cytochrome oxidase and ADP carrier play a central role in the regulation of heart energy metabolism. The observed effect of acetyl-L-carnitine on the activity of these two protein systems as well as on that of phosphate translocator [8], may explain, at least in part, the stimulatory effect of this compound on cellular energy metabolism in aged animals.

Acknowledgments: This work was accomplished with funds from Ministero Pubblica Istruzione (60%), Progetto Finalizzato Invecchiamento (C.N.R.) and from Sigma-Tau Company. The authors acknowledge the excellent technical assistance of Mr. F. Fracasso and L. Gargano.

References

- [1] Hansford, R.G. (1983) *Biochim. Biophys. Acta* 726, 41–80.
- [2] Fritz, I., Schultz, S. and Srere, P. (1963) *J. Biol. Chem.* 238, 2509–2517.
- [3] Siliprandi, N., Siliprandi, D. and Cimen, M. (1965) *Biochem. J.* 96, 777–780.
- [4] Bremer, J. (1962) *J. Biol. Chem.* 237, 3628–3632.
- [5] Gadaleta, M.N., Petruzzella, V., Renis, M., Fracasso, F. and Cantatore, P. (1990) *Eur. J. Biochem.* 187, 501–506.
- [6] Ruggiero, F.M., Cafagna, F., Gadaleta, M.N. and Quagliariello, E. (1990) *Biochim. Biophys. Res. Commun.* 170, 621–626.
- [7] Curti, D., Dagani, M.R., Galmozzi, M.R. and Marzatico, F. (1989) *Mech. Ageing Dev.* 47, 39–45.
- [8] Paradies, G., Ruggiero, F.M., Gadaleta, M.N. and Quagliariello, E. (1992) *Biochim. Biophys. Acta* 1103, 324–326.
- [9] Fry, M., Blondin, G.A. and Green, D.E. (1980) *J. Biol. Chem.* 255, 9967–9970.
- [10] Robinson, N.C., Strey, F. and Talbert, L. (1980) *Biochemistry* 19, 3656–3661.
- [11] Abramovitch, D.A., Marsh, D. and Powell, G.L. (1990) *Biochim. Biophys. Acta* 1020, 34–42.
- [12] Kramer, R. and Klingenberg, M. (1980) *FEBS Lett.* 119, 257–260.
- [13] Hoffmann, B., Stockl, A., Schlame, M., Beyer, K. and Klingenberg, M. (1994) *J. Biol. Chem.* 269, 1940–1944.
- [14] Paradies, G. and Ruggiero, F.M. (1990) *Biochim. Biophys. Acta* 1016, 207–212.
- [15] Paradies, G., Ruggiero, F.M., Dinoi, P., Petrosillo, G. and Quagliariello, E. (1993) *Arch. Biochem. Biophys.* 307, 91–95.
- [16] Doussiere, J., Ligeti, E., Brandolin, G. and Vignais, P.V. (1984) *Biochim. Biophys. Acta* 766, 492–500.
- [17] Klingenberg, M. and Appel, M. (1980) *FEBS Lett.* 119, 195–199.
- [18] Block, M.R., Pougeois, R. and Vignais, P.V. (1980) *FEBS Lett.* 117, 335–340.
- [19] Nohl, H. and Kramer, R. (1980) *Mech. Ageing Dev.* 14, 137–144.
- [20] Kim, J.H., Shrago, E. and Elson, C.E. (1988) *Mech. Ageing Dev.* 46, 279–290.
- [21] Hostetler, K.J. (1991) *Biochim. Biophys. Acta* 1086, 139–140.