CHANGES IN MONOAMINE OXIDASE ACTIVITY ASSOCIATED WITH THE UNCOUPLING OF RAT LIVER MITOCHONDRIA

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Received 5 September 1980
Revised version received 3 October 1980

1. Introduction

Monoamine oxidase (MAO) (EC 1.4.3.4) is tightly bound to the outer membrane of mitochondria [1] and vigorous methods are required for its solubilisation [2,3]. Although such methods have allowed the characterisation of MAO the soluble enzyme shows differing responses to heat pretreatment and inhibitors dependent upon the amount of phospholipid it binds [4] and changes in reaction mechanism have been reported upon solubilisation of the enzyme [5]. The use of mitochondrial outer membrane preparations, although preserving the lipid environment of the MAO, may not, however, adequately reflect the behaviour of MAO in situ in intact mitochondria and it is likely that further consideration of the enzymes environment may be necessary. It had been reported that the respiratory state of bioenergetically competent mitochondria can markedly alter the associated MAO activity [6]. Here, changes in MAO activity related to the uncoupling of mitochondrial oxidative phosphorylation are described.

2. Materials and methods

Tyramine was obtained from Koch Light; [*side chain* 2-^{14}C]tyramine was from The Radiochemical Centre, Amersham. All other chemicals were obtained from BDH Chemicals and were of analytical grade.

Mitochondria were isolated as in [6]. When frozen preparations were required the mitochondria were frozen in liquid nitrogen and rapidly warmed to 0°C just prior to use. Protein was determined by a biuret method [7].

The incubation of mitochondria and the subsequent sampling for the assay of MAO activity using [^{14}C]tyramine as substrate were as in [6] in a medium containing 120 mM sucrose, 5 mM $MgCl_2$ and 10 mM potassium phosphate (pH 7).

The time at which uncoupling occurred was determined using a Rank oxygen electrode to determine the Respiratory Control Quotient [8] of the preparations after incubation under state 1 conditions [9] for various times.

3. Results

Mitochondria incubated under respiratory state I conditions (no exogenous respiratory substrate or ADP) had an initial low MAO activity which subsequently increased on incubation at 30°C to an activity ~10-fold greater [6]. The time of incubation at 30°C required for such changes in activity to occur was found to vary from preparation to preparation and to be markedly reduced by ageing of the preparation at 0°C for several hours. Mitochondrial oxidative phosphorylation can also be impaired by prolonged incubation under state I conditions and by ageing at 0°C and as the data in fig.1 indicates a good correlation was found between the time of incubation required to cause uncoupling and the time needed to produce a doubling of the MAO activity.

The temperature dependence of this transition in MAO activity is shown in fig.2 in which mitochondria were uncoupled by freezing and thawing [10] just prior to incubation at the indicated temperature. It can be seen that although the mitochondria were

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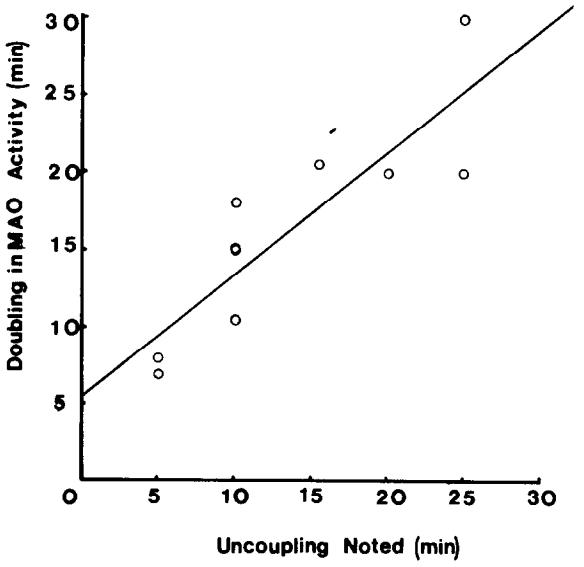

Fig.1. Relationship between uncoupling and the increase in MAO activity. For each of 10 separate preparations the time at which the MAO activity increased to double the initial level is plotted against the time of state I incubation required to cause uncoupling. The regression line for the data is drawn ($r = 0.875, p < 0.001$ ($n = 10$)).

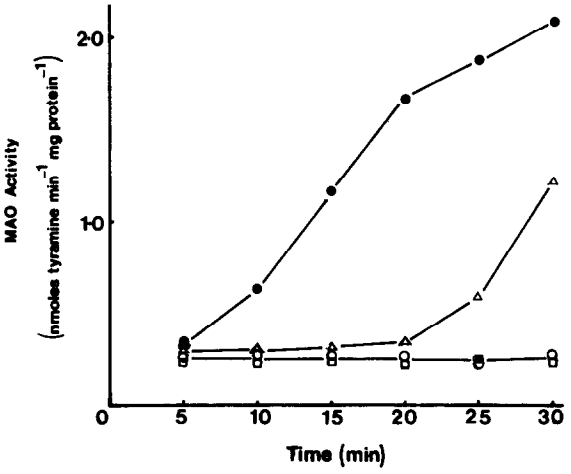

Fig.2. Effect of temperature on the transition in MAO activity. At zero time 0.05 ml frozen thawed mitochondria (100 mg protein/ml) was added to 2.95 ml incubation medium equilibrated to the various temperatures. Samples (0.4 ml) were taken at the times indicated and rapidly warmed to 30°C and assayed for MAO activity: 30°C (●); 20°C (△); 10°C (○); 0°C (□).

uncoupled in each case by freezing and thawing just before starting the incubations the transition in MAO activity was retarded at 20°C and did not occur at all during 30 min incubation at 0°C or 10°C. The MAO activity of mitochondria was determined at various initial tyramine concentrations from 20 μM to 1 mM both before and after the transition in activity had occurred. Such data allowed the calculation of the kinetic constants for the enzyme under both conditions and were determined to be:

(i) Before the transition $K_m = 318$ μM, $V_{max} = 0.81$ nmol tyramine . min^{-1} . mg $protein^{-1}$;

(ii) After the transition $K_m = 237$ μM, $V_{max} = 8.6$ nmol tyramine . min^{-1} . mg $protein^{-1}$.

In an attempt to investigate further the relationship between uncoupling and MAO activity the uncoupling agent 2,4-dinitrophenol (2,4-DNP) was employed. The transition in MAO activity could be promoted by the addition of 2,4-DNP to mitochondria after 9 min incubation (fig.3a). If added at the beginning of the incubation however 2,4-DNP prevented increase in MAO activity during the subsequent 30 min. However, similar effects of this uncoupling agent were found for mitochondria pre-uncoupled by freezing and thawing (fig.3b).

4. Discussion

The MAO activity of coupled mitochondria incubated under respiratory state I conditions was always

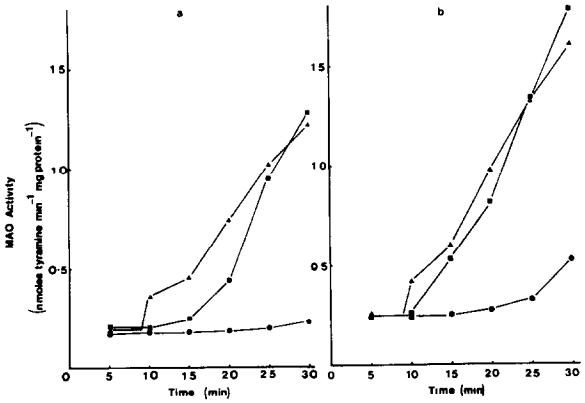

Fig.3. Effect of 2,4-DNP on the transition in MAO activity. Incubations were started by the addition of 0.05 ml of (a) freshly prepared mitochondria or (b) frozen thawed mitochondria (both 100 mg protein/ml) to 2.95 ml incubation medium and assayed for MAO activity at the times indicated. No addition (■); 2,4-DNP (166 μM final conc.) $t = 0$ (●) or $t = 9$ (▲).

found to be appreciably lower than the activity after prolonged incubation at 30°C and as the data in fig.1 show the onset of the increase in MAO activity observed during such incubations can be correlated with the time at which uncoupling occurred. The large increase in MAO activity observed after the mitochondria became uncoupled was found to be due to a 10-fold increase in the enzymes V_{max}, the change in the estimated value of K_m not being significant even at the 10% confidence level.

The transition in MAO activity of mitochondria uncoupled by freezing and thawing was found to be temperature sensitive (fig.2), with the transition not occurring at all during 30 min incubation at 0°C or 10°C and occurring less rapidly at 20°C than 30°C. This temperature sensitivity of the transition and the slow rate of increase in activity even at 30°C suggests that the change in MAO activity may be due to some kind of reorientation of the enzyme in the lipid phase of the membrane subsequent to the uncoupling event; the reorientation being slower or not happening at all at lower temperatures due to the more crystalline nature of the lipid phase. It has been postulated [11] that such intimate lipid–protein interactions could alter the binding site of MAO.

The use of the protonophore 2,4-DNP to uncouple the mitochondria during state I incubations produced a varied effect on the transition in MAO activity dependent upon the time of the addition (fig.3a) and similar effects were observed with frozen thawed mitochondria (fig.3b). It should be noted however that the transition in MAO activity normally observed in mitochondria uncoupled by freezing and thawing prior to incubation can be prevented by the inclusion of 2,4-DNP at the start of the incubation but 2,4-DNP enhanced the change when added after 9 min incubation (fig.3b) indicating that 2,4-DNP can in some way interfere with the transition in MAO activity.

The uncoupling action of protonophores is due to the solubility of the protonated molecule in membranes which facilitates the flux of protons through the otherwise impermeable inner membrane [12]. This solubility, however, will not be restricted to the inner membrane and consequently the uncoupling agent will also be present in the outer mitochondrial membrane. The addition of uncoupling agents to membranes is known to be accompanied by changes in the mobility of both membrane lipids and proteins [13] and such changes in mobility may be responsible for the effects of 2,4-DNP on the transition in MAO activity observed for both freshly isolated mitochondria and those uncoupled by freezing and thawing prior to incubation. Thus it would seem reasonable to propose that 2,4-DNP in addition to its uncoupling action, is capable of interfering with the transition in MAO activity in this manner.

This work demonstrates that the MAO activity of mitochondria can be markedly influenced by the stability of mitochondrial coupling which in addition to the sensitivity of MAO to the respiratory state of the mitochondria demonstrated in [6] underlies the need for the use of bioenergetically competent mitochondria under defined conditions in the study of MAO in order that the in vivo environment of the enzyme on the outer mitochondrial membrane may be more accurately reproduced.

Acknowledgements

I am indebted to Dr R. A. Reid for his advice during this work. Part of this work which was supported by a CASE award (SRC and ICI) was performed in the laboratories of ICI Pharmaceuticals Division under the supervision of Dr D. N. Middlemiss.

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