MITOCHONDRIAL STRUCTURE AS A CONTROLLING FACTOR OF MONOAMINE-OXIDASE ACTIVITY AND THE ACTION OF AMINO-OXIDASE-INHIBITORS*

H. AEBI

Medizinisch-chemisches Institut der Universität Bern

IN THE cell, enzyme activity is mainly limited by the steady state substrate concentration, which is a resultant of substrate formation, utilization and penetration. Under certain experimental conditions this is also true for particle bound monoamine oxidase (MAO) activity. In order to get some information on the nature of the rate limiting factor in overall MAO activity, experiments have been made with (A) purified MAO preparations (from dog kidney) and (B) mitochondrial fractions from rat liver at different levels of structural and functional integrity. The following activities have been measured: (1) NH_3 production by Seligson's method, (2) total O_2 consumption by means of Warburg's direct method, (3) rate of H_2O_2 production, using the coupled oxidation of ^{14}C-formate as an indicator reaction, and (4) the aldehyde formation by semicarbazide trapping.

MAO is one of the yellow enzymes which react directly with O_2 thereby producing H_2O_2. In the presence of excess catalase and of a suitable H-donor (such as formate) for each mole of substrate (tyramine) one mole of ammonia is produced and an equivalent quantity of H-donor is oxidized peroxidatically:

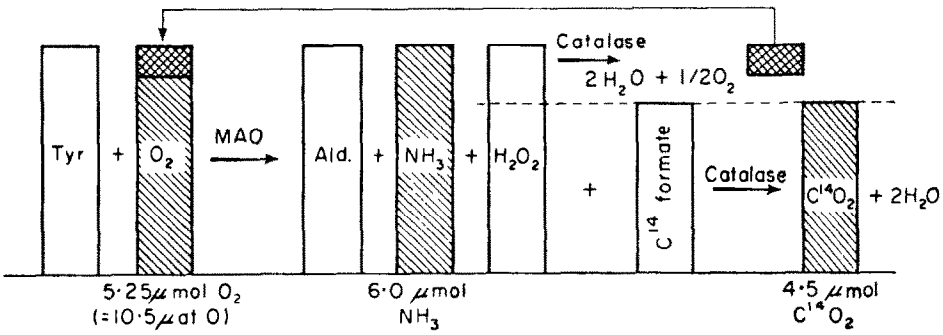
$$\boxed{R}-CH_2-NH_2 + O_2 + H_2O \longrightarrow \boxed{R}-C\begin{matrix}=O\\ -H\end{matrix} + NH_3 + H_2O_2$$

$$\boxed{Donor}\begin{matrix}H\\ H\end{matrix} + H_2O_2 \longrightarrow \boxed{Donor} + 2H_2O$$

Thus, theoretically, in such a system the molar ratio $O_2 : NH_3$: donor = 1 : 1 : 1. As an example, the results of experiments done with a stabilized MAO preparation are given in Fig. 1. From this it can be seen that if 200 µg crystalline catalase are added per flask about 75% of the H_2O_2 is used peroxidatically for coupled oxidation of ^{14}C-formate

* This investigation has been made in collaboration with Drs. A. Hassan and F. Stocker.

and 25% is decomposed catalatically. Provided that MAO is present in solubilized form the percentage of H_2O_2 used for coupled oxidation is a function of catalase concentration. If the latter is varied, a saturation curve is observed, levelling off at very high catalase concentrations (> 1 mg/flask) only.



Total of reaction products formed: 6·0 µmol NH_3 and 6·0 µmol H_2O_2

Fraction of H_2O_2 decomposed catalatically: 6·0 − 4·5 = 1·5 µmol ~ 25%

Fraction of H_2O_2 used peroxidatically: 4·5 µmol ~ 75%

Mean values of 5 experiments with MAO-preparation of dog kidney
(C. Giordano, J. Bloom and J. P. Merill Experientia 16, 346–47, 1960)

FIG. 1. MAO activity of a lyophilized preparation from dog kidney with respect to O_2 consumption, and NH_3 and $^{14}CO_2$ production. Composition of the medium (total volume 2·5 ml.): 36 mg MAO preparation (= 10 mg biuret-protein), 200 µg crystalline horse liver catalase ("Boehringer"), 25 µmole semicarbazide and 0·5 ml M/15 sodium phosphate buffer pH = 7·0 in the main compartment; 25 µmole tyramine (pH 7·0) and 80 µmole ^{14}C-formate in first side arm, 0·2 ml. N HCl in second side arm; 0·2 ml 20% NaOH in the central cup. Gas-phase: O_2. Warburg flasks incubated for 10 + 60 min at 37°. Reaction started by tipping in tyramine and ^{14}C-formate; reaction stopped by tipping in HCl from second side arm.

Suspensions of freshly prepared rat liver mitochondria behave in a similar way as far as the ratio of peroxidatic/catalatic action is concerned. Their MAO activity, however, largely depends on experimental conditions. When compared with lysed mitochondria — as used usually — intact mitochondria suspended in isotonic saccharose and incubated in the presence of pyruvate and tyramine show a much smaller NH_3 production and H_2O_2 formation, whereas their O_2 consumption is much higher. In the experiment presented in Fig. 2 and 3 two different functional states are compared: (a) Mitochondria incubated in a medium which enables them to maintain more or less their structural integrity.

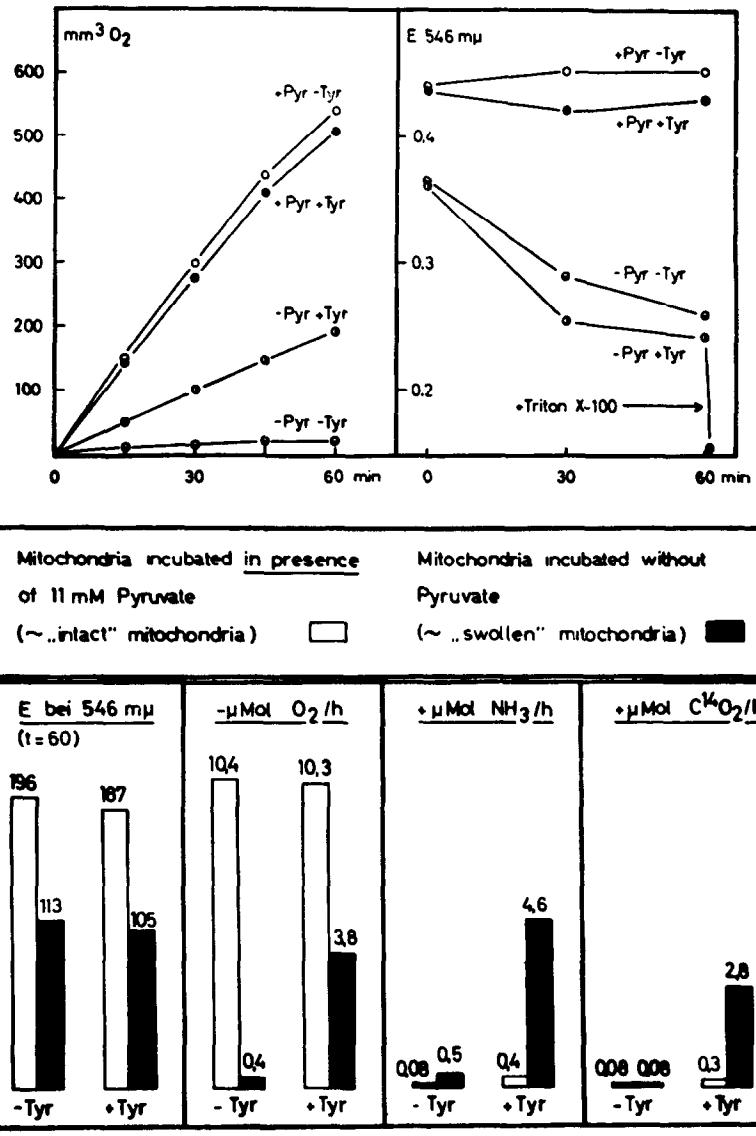


FIG. 2 and 3. Effect of pyruvate addition on MAO activity, overall oxidation rate and optical density of a suspension of isolated rat liver mitochondria. Composition of the medium (total volume 2·5 ml.) : 1 ml. of mitochondrial suspension in 0·25 M saccharose (= 20–25 mg biuret-protein) obtained by Leuthardt's method; catalase-solution containing 500 μg of the pure enzyme; 27 μmole Na-pyruvate, 5 μmole ATP, 25 μmole Komplexon III, isotonic KCl, $MgCl_2$ and phosphate, pH = 7·0 up to volume. In the side arm 25 μmole tyramine (pH 7·0) and 80 μmole ^{14}C-formate. Gas phase: air. Warburg flasks incubated for 15 + 60 min at 37°. Measurement of the optical density made immediately after the end of experimental period by diluting 0·1 ml. of the batch in 5 ml. cold 0·25 M saccharose; extinction at 546 mμ of the diluted suspension read within one minute.

This state is accompanied by a high O_2 consumption and a high optical density of the mitochondrial suspension throughout the incubatio period. (b) Mitochondria incubated without pyruvate. This results in decreased O_2 consumption and decreasing values for optical density. These particules may be considered as "swollen" but not completely ruptured, since there is still an additional decrease in optical density after completion of lysis with Triton X-100 (see Fig. 2). Whereas there is little difference in MAO activity between the "swollen" mitochondria and a preparation of lysed particules, there is a considerable one, if the functional states resulting under conditions (a) and (b) are compared. The results given

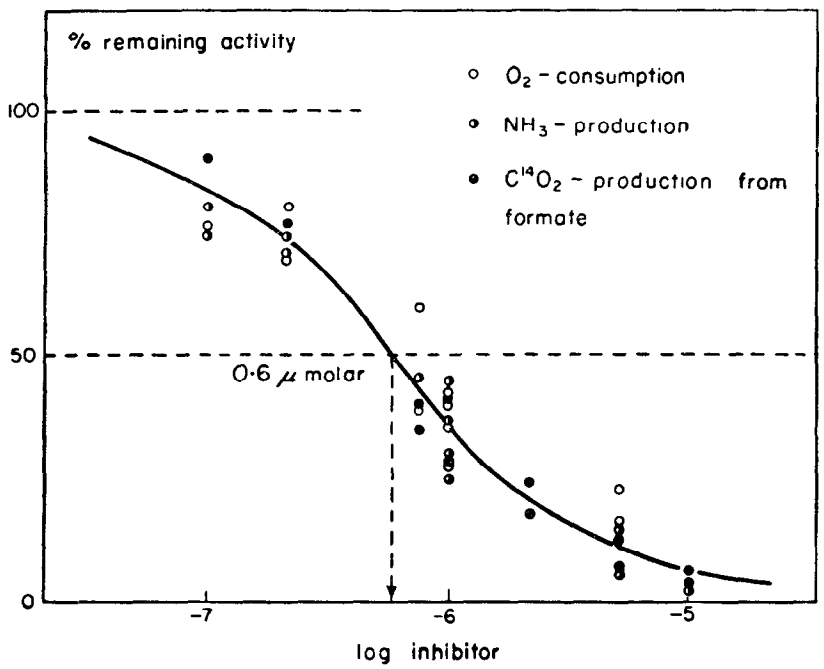


FIG. 4. Inhibition of O_2 consumption, NH_3 formation and coupled ^{14}C-formate oxidation by cisphenylcyclopropylamine in a suspension of isolated rat liver mitochondria. Experimental conditions similar to those described in Fig. 5 except that the inhibitor was added to the main compartment before tyramine and ^{14}C-formate were tipped in. The particules incubated at these conditions have to be considered as "swollen".

in Fig. 3 indicate that there is a marked difference with respect to NH_3 production and to formate oxidation in (a) apparently intact and (b) less dense, i. e. "swollen" particules. Under the experimental conditions chosen both NH_3 and $^{14}CO_2$ production are only 10% as great in the presence of pyruvate as in its absence.

Similar, but less pronounced discrepancies are observed, if the osmotic pressure of the medium is varied, if the mitochondrial suspen-

sion is frozen before incubation, or if a detergent e. g. 0·1% Triton-X-100 is added. In all these instances there is reciprocity between the rate of pyruvate oxidation (requiring intact mitochondrial structure) and MAO activity, as measured by NH_3 and $^{14}CO_2$ production (both activities being controlled by structural factors). This apparent dependency of particule-bound MAO activity on environmental conditions may be due to the fact, that in intact mitochondria MAO is less accessible for the substrate, or it may be due to some competitive mechanism within the respiratory chain.

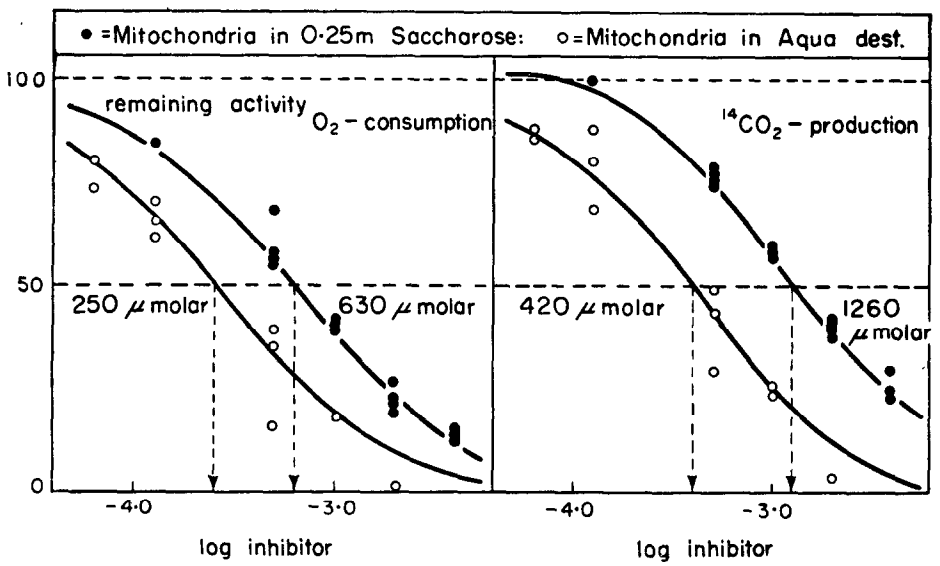


FIG. 5. Inhibition by Iproniazid of MAO activity in rat liver mitochondria incubated under different conditions. Composition of the medium (total volume 2·5 ml.): 1 ml. mitochondrial suspension in distilled water or in 0·25 M saccharose, 100 μg crystalline, catalase, 20 μmole pyruvate, 25 μmole Komplexon III, 2·5 μmole ATP, 25 μmole semicarbazide, 60 μmole phosphate buffer (pH = 6·8) KCl, $MgCl_2$ and 40 μmole ^{14}C-formate in the main compartment; 25 μmole tyramine and various amounts of Iproniazid in the side arm; 0·2 ml. 20% NaOH in the central cup. Gas phase: air. Incubation period 15 + 60 min. Activities observed in absence of inhibitor = 100%.

The activity of MAO inhibitors, which depends on a variety of experimental factors, is also influenced by the structural state of the mitochondria. In the presence of increasing amounts of MAO inhibitors all the activities mentioned above are reduced to the same extent; this is also true for coupled formate oxidation, provided that excess catalase is present (Fig. 4). If no excess catalase has been added and, therefore, only a small fraction of H_2O_2 is used for coupled oxidation the I_{50} value for $^{14}CO_2$ formation is a 2–3 fold greater than those for O_2 consumption

and NH_3 formation. The I_{50} values measured in rat liver mitochondria depend on the structural state of the particules, especially if substrate and inhibitor are added simultaneously. In this respect Iproniazid behaves as follows: O_2 consumption and NH_3 production of particules lysed in distilled water are inhibited 50% in the presence of $2{\cdot}5 \times 10^{-4}$ M inhibitor tipped in together with substrate. On the other hand an approximate 3-fold greater inhibitor concentration (about 7×10^{-4} M) is required if the mitochondrial suspension is incubated in 0·25 M sacharose, i. e. at conditions that essentially antagonize disintegration. As shown in Fig. 5, this difference appears, whatever parameter for MAO activity is taken. It must be mentioned, however, that the more structural integrity can be maintained by optimal incubation conditions, the less is MAO activity and the less effective becomes additional catalase. That is why the experimental procedure in the inhibitor tests (Fig. 5) differs somewhat from that adopted in the experiment in Fig. 2 and 3. A shift in I_{50} values can also be observed if the mitochondrial suspension (in 0·25 M saccharose) is frozen previously or treated with 0·1% Triton-X-100.

The structural dependency of MAO activity and of the action of MAO inhibitors may be of significance for any study of pharmacological effects at the subcellular level. In view of these results* it is very difficult to extrapolate figures for an enzymatic activity obtained from lysed preparations to the intact cell, if this enzyme is structurally bound. The assumption that substrate saturation of an enzyme is only exceptionally reached *in vivo* and in the intact cell may also be true for subcellular fractions. It seems that this is the case for mitochondrial MAO activity (which represents about 2/3 of total MAO activity in rat liver, according to Dr. De Duve) and that this is possibly due to limited accessibility for the substrate or some other reactant.

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* For details consult:

(a) H. AEBI, I. QUITT and A. HASSAN: Uricase, Xanthinoxydase und Monoaminoxydase als H_2O_2 — Donoren peroxydatischer Umsetzungen; *Helv. Physiol. Acta* 1962 (in press).

(b) H. AEBI and F. STOCKER: Abhängigkeit der Monoaminoxydase — Aktivität und peroxydatischer Umsetzungen von der Mitochondrien struktur; *Biochem. Z.* 1962 (in press).