Oxidation of synephrine by type A and type B monoamine oxidase

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Summary. Synephrine (SP) was found to be a substrate for monoamine oxidase (MAO) in rat brain mitochondria, showing the K_m and V_{max} values of 250 μM and 32.6 nmoles/mg of protein/30 min respectively. The inhibition studies showed that the SP oxidation was carried out by both type A and type B MAO and a major part of the activity was due to type A MAO.

Mitochondrial monoamine oxidase [amine: oxygen oxidoreductase (deaminating, flavin-containing); EC 1.4.3.4] (MAO) is believed to exist in many animal tissues in 2 functional forms called type A and type B MAO[2-4]. Type A MAO has been shown to be active with 5-hydroxytryptamine and norepinephrine as substrates, and sensitive to the inhibition by a low concentration of clorgyline. Type B MAO has been shown to be active with β-phenylethylamine and benzylamine, and sensitive to the inhibition by a low concentration of deprenyl. Some substrates, such as kynuramine, tyramine, tryptamine and dopamine, are oxidized by either type of MAO. It should be noted that the concept of the 2 types of MAO is primarily based on the sensitivity to clorgyline[2].

Recently, trace amines such as β-phenylethylamine, tyramine and octopamine, which are present normally in the central nervous system, are the objects of growing interest[5]. The metabolic pathways of the trace amines are now being actively explored. Synephrine (SP), the N-methylated compound from octopamine, was identified in human urine[6,7], and it was found that SP was synthesized from octopamine in vivo in rat brain[8]. Although SP is easily expected to be a substrate for MAO, to our knowledge, such a study has never been carried out. Therefore, the present study deals with the oxidation of SP by type A and type B MAO.

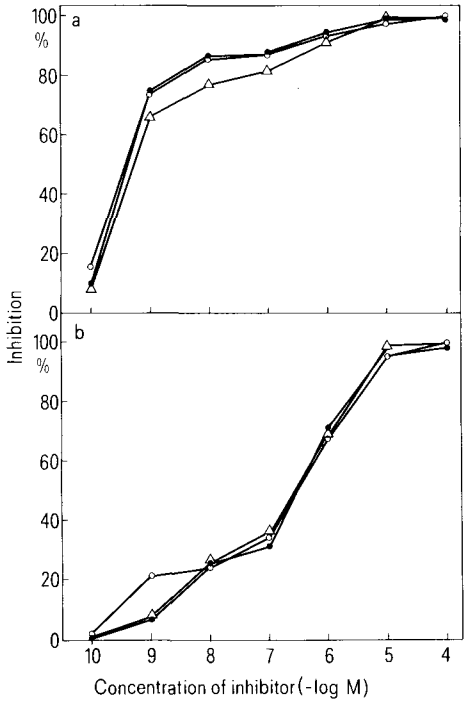
Materials and methods. A crude mitochondrial fraction was isolated from whole brains of male Sprague-Dawley rats weighing 100–150 g as described previously[9]. MAO activities were determined fluorometrically by the method of Guilbault et al.[10,11]. For each assay, 0.20–0.41 mg of mitochondrial protein was used. The assays were carried out at 37 °C and pH 7.4 for 30 or 60 min. Care was taken not to convert more than 20% of the substrate to reaction product. For the kinetic analyses, substrate concentrations over the range of 0.2–5.0 mM were used. Clorgyline, a selective inhibitor of type A MAO[2], was generously supplied by May & Baker Ltd., Dagenham, England. Deprenyl, a selective inhibitor of type B MAO[12], was kindly donated by Prof. J. Knoll, Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary. It was confirmed that each inhibitor neither interfered with the formation of fluorescent compounds nor quenched their fluorescence when hydrogen peroxide was added directly. The mixture was preincubated with each inhibitor at 37 °C for 10 min to ensure maximal enzyme inhibition. Protein was measured by the conventional biuret method.

Results and discussion. SP was a substrate for MAO. The Michaelis-Menten kinetic constants for SP were determined from the Lineweaver-Burk plots: the K_m and V_{max} were 250 μM and 32.6 nmoles/mg of protein/30 min, respectively.

In order to determine the specific type of MAO involved in the SP metabolism, the sensitivity to clorgyline and deprenyl was studied. Since we reported that the sensitivity to both inhibitors was affected by substrate concentration when β-phenylethylamine and phenylethanolamine were used as substrates[13], we tested 3 different concentrations of SP, viz., 25.0, 250 and 5000 μM. As can be seen in the figure, in the curves with clorgyline, clear plateaus appeared at 10^{-8}–10^{-7} M. MAO activity with 5000 μM SP was slightly more resistant to clorgyline than those with the lower substrate concentrations. In the curves with deprenyl, plateaus were also observed at 10^{-8}–10^{-7} M; there were no significant differences in inhibition pattern with deprenyl among the 3 concentrations. These results indicate that SP is deaminated by both types of MAO over a wide concentration range of the substrate and a major part of the activity is due to type A enzyme.

In the previous paper[13], we reported extremely similar inhibition patterns with octopamine, the precursor of SP, to those with SP in the present study. Therefore, our present results imply that the N-methylation of a monoamine does not result in any change in substrate preference. In this connection, it should be recalled that norepinephrine, epinephrine (the N-methylated derivative of norepinephrine), normetanephrine and metanephrine (the N-methylated derivative of normetanephrine) were equally oxidized exclusively by type A MAO[14].

In the present communication, we have clarified the nature of SP as substrate for type A and type B MAO. Although the physiological significance of SP in the brain has not been well defined, our present observations may serve to elucidate the in vivo dynamics of SP in the brain in future.



Inhibition of MAO in rat brain mitochondria by clorgyline (a) and deprenyl (b) using various concentrations of synephrine as substrate. The concentrations of the substrate were 25.0 (○——○), 250 (●——●) and 5000 (△——△) μM. Each point represents the mean obtained from duplicate determinations.

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Conformation of ribonuclease S-protein

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Summary. Ribonuclease S-protein exhibits a pH-dependent conformational transition between folded and unfolded states, and some unfolded S-protein persists up to pH 8. The histidine C2 proton resonance of the unfolded species was erroneously assigned by Bradbury et al.[1] to histidine residue 119 of the folded species.

Bradbury et al.[1] have attempted to explain our earlier observations[2] on the histidine residues of ribonuclease S-protein by invoking an 'irreversible denaturation' of S-protein, although they cited no evidence to support this assertion. Also, the peak which we had assigned to unfolded (or denatured) S-protein they assigned to histidine residue 119 of the folded S-protein. A test of which assignment is correct can be made on the basis of the behavior of this resonance under different experimental conditions.

In figure 1 are shown spectra of samples of S-protein at different pH values. It is clear that the peak labelled with the arrow increases in relative area as the pH is lowered, until at pH < 2 it corresponds to the equivalent histidine residues of unfolded S-protein. In order to confirm that this peak corresponded to unfolded or denatured S-protein at higher pH values, where significant amounts of folded S-protein are present, we heated a sample and added guanidine hydrochloride (^2H_2O-lyophilized) to another (figure 2). In both cases this peak clearly increased in area at the expense of other resonances, as expected for an unfolding process[5]. Consequently this must be the resonance (H-u) of the equivalent histidines of unfolded S-protein.

It might be argued that this resonance could also correspond to His 119 of the folded species if it happens to coincide under normal pH and temperature conditions with that of the unfolded material; this would imply that His 119 is in an essentially denatured environment in the globular S-protein. This seems unlikely since at pH values > 6 we observed 3 other resonances (figure 1), corresponding to the 3 histidine residues present in S-protein. 2 of these are sufficiently close (about 0.1 ppm) that they could not be resolved at the lower observing frequency (100 MHz) used by Bradbury et al.[1].

2 of these resonances were readily assigned[2] to His residues 48 and 105 on the basis of their near identity with resonances in spectra of ribonuclease A and S[6]. The third resonance, that of His 119, was the only resonance affected by the addition of Pi to the solution[2]. In fact, the most downfield peak in the spectra of S-protein at pH < 6 is always larger than that assigned to His 48 (figure 1), and consequently must correspond to 2 histidine residues.

The other peak, which varies significantly in area, we now confirm our assignment to unfolded material (H-u), since clearly the amount of this material depends upon the conditions. This resonance is a significant feature of spectra at all pH-values shown by Bradbury et al.[1], and we must conclude that there is some unfolded material present in all their S-protein samples. The value of '30%' estimated by Bradbury et al. for the amount of denatured material was derived from the relative area of this resonance found by us at pH 4.6, since we could not satisfactorily fit spectra quantitatively above this value[2]. In fact, a comparison of our spectra of S-protein with that of Bradbury et al.[1] at pH 4.5 (figure 9 of the cited paper) shows a remarkable consistency.

Bradbury et al.[1] concluded that His 48 in S-protein 'titrates normally'. This is not borne out by their results, as well as ours, since a) the resonance of His 48 is broadened, especially at pH > 6, so that it was sometimes not possible to follow it, and b) its titration curve is shifted upfield relative to that of a normal histidine residue[1,2]. In fact, except for the absence of the low pH inflection, this curve is remarkably similar to that of His 48 in ribonuclease A or S[7], as we previously pointed out[2]. Consequently there is no basis for the statement of Bradbury et al.[1] that His 48 is 'accessible to solvent', and they quote no evidence for this other than their incorrect assertion that its titration curve is 'normal'. Thus, there is no apparent basis for the distinction between their conclusion, that 'the relative orientation of the C2 proton of His 48 and the ring of Tyr 25 remain about the same as in RNase-A'[1], and the conclusion they attribute to us of a 'highly structured environment of His-48'.

We have carefully referred to the material giving rise to the extra titrating C2 peak in NMR-spectra of S-protein as unfolded rather than denatured[2] since the term 'native' hardly applies to S-protein. However, the likelihood that this material is 'irreversibly denatured', as Bradbury et al.[1] assert can be discounted for the following reasons: a) spectra of S-protein were reversible as a function of pH (figure 1); b) the resonance which Bradbury et al.[1] assigned to His 119 in fact arises from unfolded material, and would indicate that they had as much 'denatured' S-protein in their sample as we had in our samples; c) if there was as