Molecular Properties of the Monoamine Oxidases

by David J. Edwards

Abstract

To understand the biological significance of a decreased platelet MAO activity in schizophrenia, it is important to answer many questions about the basic molecular properties of the enzyme. The past decade has witnessed a huge expansion of research in this area which has provided some of these answers. A wide variety of experimental evidence strongly supports the concept of two forms of MAO (types A and B) that are present in most tissues but in varying relative amounts. However, human platelets contain only the type B form of MAO. This review discusses what is known about the molecular properties of MAO, what questions remain unresolved, and the significance of both the answered and unanswered questions to understanding the measurement of platelet MAO activity in schizophrenic patients.

The role of neurotransmitter amines in neuronal function is well established. Various lines of evidence also suggest that alterations in the metabolism of these amines are importantly involved in the behavioral effects caused by certain pharmacological agents and mental disorders. Monoamine oxidase (MAO) is an enzyme found in most tissues of the body. In nervous tissues, it serves to degrade several biogenic amines which are thought to act as neurotransmitters, including norepinephrine, dopamine, and serotonin, as well as various trace amines, such as octopamine, phenylethanolamine, and tyramine, whose function is not yet well understood.

Since MAO plays a key role in the metabolism of all these amines, it has long been recognized that an abnormal activity of this enzyme could produce abnormal tissue concentrations of the various amine substrates and thereby possibly account for certain mental abnormalities, such as schizophrenia.

The discovery of MAO activity in human blood platelets (Paasonen and Solatunturi 1965) suggested the possibility of using platelets as a readily obtainable source of the enzyme in studies of psychiatric disorders. This led to the work of Murphy and Wyatt (1972), who first demonstrated a reduced activity of MAO in platelets from schizophrenic patients. Numerous studies have attempted to replicate this original observation, but the results have been mixed. In a recent review of 26 studies of chronic schizophrenia, Wyatt, Potkin, and Murphy (1979) pointed out that in 19 of the studies there was a statistically significant decrease in platelet MAO activity of schizophrenics as compared to control subjects.

In view of the many studies that have shown a reduced platelet MAO activity in chronic schizophrenic patients, a number of crucial questions emerge: What, if any, is the biological significance of a decrease in platelet MAO activity? What factors contribute to a low MAO activity? Does a reduced activity of MAO in the platelet reflect a corresponding decrease in the brain and other tissues? Or, since platelets contain only the B form of MAO, is only that form of the enzyme reduced in other tissues, and not the A form, which is the one mainly responsible for norepinephrine and serotonin catabolism? Since platelet MAO ac-
tivity is at least in part under genetic control (Murphy 1976), it is important to know what are the molecular differences between the A and B forms of the enzyme and whether they are under the control of the same or different genes. Is the reduced activity of platelet MAO due to a decreased synthesis of protein (i.e., gene product), or is a normal amount of the enzyme produced but with an altered catalytic function? Finally, is the reduction in MAO activity (whether it occurs just in platelets or in other tissues as well) sufficient to alter the metabolism of any of the biogenic amines metabolized by MAO, or is a reduced platelet MAO activity only important as a biological marker of schizophrenia?

Before these questions can be answered, an understanding of the basic molecular properties of MAO is needed. Although there are many unanswered questions about the structure and function of this enzyme, rapid progress is being made, as evident from a recent volume devoted to this subject (Singer, Von Korff, and Murphy 1979). In the remainder of this article, I will discuss our current understanding of these molecular properties.

General Properties of MAO

Although it is now well recognized that there are multiple forms of MAO (see below), they nevertheless have many features in common. Whether the multiple forms of MAO represent different proteins or different modifications of a single protein, there clearly are more similarities than dissimilarities between them.

MAO catalyzes the oxidative deamination of monoamines according to the following reaction:

\[ \text{RCH}_2\text{NH}_2 + \text{H}_2\text{O} + \text{O}_2 \rightarrow \text{RCHO} + \text{NH}_3 + \text{H}_2\text{O} \]

where the structure of R depends on the particular substrate.

MAOs are flavin-containing enzymes located on the outer mitochondrial membrane. They are to be distinguished from other amine oxidases, such as the plasma amine oxidase and diamine oxidase. In contrast to MAO, the latter enzymes are soluble, are inhibited by semicarbazide, and do not contain flavin.

Attempts to gain information about the structure of MAO have been hindered because it is tightly bound to the mitochondrial membrane. Since some of the properties of the enzyme may be dependent upon not only the structure of the protein but also on its environment in the lipophilic membrane (Youdim 1975b), studies using solubilized enzymes may obscure some of its key structural properties. This may be particularly important in trying to determine the structural basis for the multiple forms of the enzyme. On the other hand, purification of the enzyme, and hence its solubilization from the membrane components, is essential to understanding the structural details that determine the enzyme’s catalytic properties.

Methods for the purification of MAO have been reviewed by Youdim (1975a). Generally, these procedures involve isolation of mitochondria from tissue homogenates, followed by solubilization of the enzyme and finally purification by standard techniques of protein purification. Solubilization is usually carried out by treatment with nonionic detergents (such as Triton X-100), sonication, or a combination of both. The best of these procedures yields preparations of MAO obtained from rat brain or liver or beef liver which have specific activities of about 100-fold higher than the intact mitochondria (McCauley 1978; Salach et al. 1976; Youdim 1975a). A purification of pig brain MAO resulted in an enzyme which had a specific activity 1,200-fold higher than the mitochondria (Salach et al. 1976). The appearance of only a single protein band on polyacrylamide gel electrophoresis run in the presence of sodium dodecyl sulfate indicated that the purified enzyme was homogeneous.

Purified rat liver MAO has been found to contain approximately one mole of covalently bound flavin per 120,000 g of protein (Youdim 1976). There are apparently two subunits of equal size for each 120,000 dalton protein, but only one of the subunits contains flavin. The mode of attachment of the covalently bound flavin was elucidated by Kearney et al. (1971), who isolated the flavin-containing pentapeptide from beef liver MAO. The sequence of this peptide was determined to be Ser-Gly-Gly-Cys-Tyr. The flavin was attached via a thioether linkage between the 8α position of the riboflavin moiety to the cysteine.

The propargylamine inhibitors of MAO, including pargyline, clorgyline, and deprenyl, are thought to inactivate MAO by forming a covalent bond at the N-5 position of the flavin (Maycock et al. 1976).

Iron is also thought to have a functional role in MAO activity, but the exact site at which it is important has not been determined. It may act as a prosthetic group, or it may be involved in either the synthesis of the apoenzyme or the incorporation of the flavin (Youdim 1976). Both iron and riboflavin deficiencies have been reported to result in a decrease in MAO activity (Sourkes and Missala 1976). However, it is unclear to what extent moderate variations in the intake of these nutritional factors affect MAO activity.
Multiple Forms of MAO

Before the late 1960s, MAO was generally regarded as a single enzyme. Little attention was paid to the possibility of multiple enzyme forms, although several bits of indirect evidence suggested their existence. For example, Oswald and Strittmatter (1963) observed a striking difference in the thermal stability in the enzyme activity deaminating serotonin and tyramine by rat liver mitochondria. Van Woert and Cotzias (1966) noted that various anions caused an inhibition of MAO activity which differed depending on whether serotonin or tyramine was used as a substrate.

Youdim and Sandler in 1967 separated up to five bands of MAO activity by polyacrylamide gel electrophoresis (see Sandler and Youdim 1972). This appeared to be the first direct evidence for multiple forms, although it did not rule out the possibility that the separated species were due to the procedures used for solubilization or isolation of the enzyme. The physiological significance of the separated forms still remains uncertain. No clear correlation has been found between the separate bands of activity and different forms of MAO demonstrated by differences in inhibitor sensitivity or substrate specificity.

The most convincing evidence for multiple forms of MAO is based on the original observation of Johnston (1968), who found that two forms could be distinguished on the basis of their sensitivity to clorgyline. When varying concentrations of this inhibitor were added to tubes containing either rat brain or liver homogenates, and the MAO activity was assayed with tyramine as substrate, biphasic inhibition curves resulted. The clorgyline-sensitive enzyme activity, which was inhibited by very low (nanomolar) concentrations of the inhibitor, was designated as type A MAO. The remaining activity, which was inhibited only by clorgyline concentrations 10³ to 10⁴ times higher, was designated as type B MAO.

Some substrates have been found to be relatively specific for either the A or B forms of MAO. The clorgyline inhibition curves obtained when these substrates are used are single sigmoid curves, indicating that the substrate is deaminated by only a single type of MAO activity. If this sigmoid curve appears at very low clorgyline concentrations, the substrate is specific for type A MAO. On the other hand, if a single sigmoid curve appears at high clorgyline concentrations, the substrate is a specific substrate for type B MAO.

Table 1 summarizes the substrate specificity of types A and B MAO toward various amines. Norepinephrine has been classified as either a type A specific substrate (Neff and Yang 1974) or a nonspecific one (White and Glassman 1977), but it is probably deaminated primarily by the type A enzyme. Besides clorgyline, other MAO inhibitors are also relatively selective for either type A or type B MAO. Lilly 51641 and harmaline are both selective inhibitors of type A MAO, whereas deprenyl is a selective inhibitor of type B MAO (Neff and Yang 1974). On the other hand, many MAO inhibitors are nonspecific with respect to types A and B MAO. This includes all the MAO inhibitors presently available for clinical use, such as phenelzine and tranylcypromine.

Not all of the evidence for the existence of two types of MAO rests on the selectivity of inhibitors. Many additional lines of evidence support the concept of two enzyme forms. Table 2 indicates some of the physicochemical and physiological properties that have been studied to further characterize differences between A and B enzymes. In virtually all of these properties, with the probable exception of electrophoretic mobility, distinct differences in the A and B enzymes have been demonstrated.

Although many arguments have been raised against the A-B concept, the bulk of evidence strongly supports it. The demonstration of multiple enzyme forms by differential thermal inactivation has been

<table>
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<th>Type A-specific substrates</th>
<th>Type B-specific substrates</th>
<th>Nonspecific substrates</th>
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<tr>
<td>Serotonin</td>
<td>Benzydamine</td>
<td>p-Tyramine</td>
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<tr>
<td>Norepinephrine (?)</td>
<td>Phenylethylamine</td>
<td>m-Tyramine</td>
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<td>Normetanephrine</td>
<td>Phenylethanolamine</td>
<td>Tryptamine</td>
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<td></td>
<td>Methylhistamine</td>
<td>Dopamine</td>
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<td></td>
<td>o-Tyramine</td>
<td>Octopamine</td>
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<td></td>
<td></td>
<td>Norepinephrine (?)</td>
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</tbody>
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Data are compiled from the following references: Edwards (1978), Hough and Domino (1979), Neff and Yang (1974), Suzuki, Oya, and Katsumata (1979), and White and Glassman (1977).
Table 2. Chemical and physiological differences in the A and B forms of MAO

<table>
<thead>
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<th>Chemical properties</th>
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<tr>
<td>Substrate specificities</td>
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<tr>
<td>Inhibitor sensitivities</td>
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<td>Thermostability</td>
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<td>pH optima</td>
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<td>Electrophoretic mobility</td>
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<td>Immunological cross-reaction</td>
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<td>Treatment with proteolytic enzymes,</td>
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<td>lipases, or chaotropic agents</td>
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<td>Species differences</td>
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<td>Organ differences</td>
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<tr>
<td>Cellular localization: subcellular</td>
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<td>localization; bulk separation (perikarya vs. glial cells); cell culture; denervation studies</td>
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<td>Development</td>
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<td>Turnover rate</td>
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criticized because the differences in thermal stabilities toward different substrates lessen or disappear when isolated mitochondria are used instead of whole tissue homogenates (Houslay and Tipton 1976). However, this effect may be explained by the buffers used to resuspend the isolated mitochondria, since the thermal stabilities of both the A and B activities in the mitochondria are identical to the same activities in the whole homogenate, provided that the mitochondria are resuspended in sucrose (Edwards, Pak, and Venetti 1979).

Another apparent inconsistency in the A-B concept is that although rat heart MAO appears to contain only the type A enzyme on the basis of clorgyline inhibition, phenylethylamine and benzylamine nevertheless serve as substrates for rat heart MAO (Lyles and Callingham 1975). However, this inconsistency is easily explained by kinetic properties of the enzymatic activity. The deamination of phenylethylamine by rat heart MAO is completely blocked by low concentrations of clorgyline but not by deprenyl. Further, the affinity of this substrate for the enzyme is low (apparent Michaelis constant, or $K_m$, is 114 $\mu$M) as compared to the high affinity which is characteristic of the B enzyme (3–5 $\mu$M) (Edwards, Pak, and Venetti 1979). Thus, these results suggest simply that instead of rat heart MAO having different enzymatic properties, phenylethylamine is deaminated as a poor substrate for the A enzyme. This is due to the fact that the A and B enzymes are not absolutely specific for the so-called “specific” substrates; therefore, when one form of the enzyme is greatly predominant, as in the case of the rat heart, it nevertheless may deaminate to some extent substrates normally selective for the other form. Indeed, in 3-week-old rats, where the A:B ratio in heart is not nearly so high as in the adult rat heart, phenylethylamine deamination is partly due to enzyme activity characteristic of the B enzyme with respect to substrate affinity and inhibitor sensitivity (Edwards, Pak, and Venetti 1979).

Another example of a “specific” substrate of one form of MAO acting as a substrate for the other form is the deamination of serotonin by human platelet MAO. Although human platelets contain only the B enzyme (based on the criteria of selective inhibitors), serotonin does serve as a substrate for human platelet MAO (Edwards and Chang 1975). However, kinetic studies show that the apparent $K_m$ for the substrate is more than 10-fold higher than is characteristic for the A enzyme. Moreover, all of the activity of human platelet MAO measured with serotonin as substrate is sensitive to inhibition by low concentrations of deprenyl but not by clorgyline. In contrast, in rabbit platelets, which contain primarily type A MAO, serotonin is deaminated just as in other tissues containing type A MAO; i.e., with a low $K_m$ and a high sensitivity of inhibition by clorgyline and not by deprenyl (Edwards and Chang 1975). These results led to the conclusion that human platelet MAO is similar in kinetic properties to type B MAO in other tissues and uses serotonin as a poor substrate.

Thus, upon close examination, some of the apparent inconsistencies concerning the concept of the A and B forms of MAO disappear. What remains unresolved, however, is the molecular basis for the multiple forms of MAO. Do they represent separate proteins, or do they result from a single gene product that gives rise to the A and B enzymes as a result of different posttranslational modifications? Whether or not the A and B enzymes represent more than one protein does not detract from the fact that they may be readily demonstrated in vitro and in vivo. Thus, their pharmacological importance is unquestionable. However, a better understanding of their molecular basis may lead to answers of such questions as the genetic control of platelet MAO and whether the A and B enzymes in other tissues are under similar or different mechanisms of control.

One remaining question is how to define the A and B enzymes. Some investigators prefer to define them in terms of the activities toward selective substrates. The problem with defining types A and B MAO activities based on serotonin and
phenylethylamine deaminating activity, respectively, is evident from the lack of absolute specificity of these enzyme species. Other investigators prefer to maintain the nomenclature of Johnston (1968) by using tyramine as a nonselective substrate and the selective inhibitors to obtain the inhibition curves. Then, the proportion of A and B enzymes is defined by the location of the plateau. However, one difficulty with this approach is that when one form is present in much smaller amounts than the other, the curves may not appear biphasic, and it may be impossible to distinguish the plateau from the baseline. Thus, we think it is better to use the selective inhibitors in combination with the selective substrates to measure the activity of the A and B enzymes (Edwards and Malsbury 1978).

Characteristics of Platelet MAO

Robinson et al. (1968) compared the substrate and inhibitor characteristics of MAO in human platelets with MAO in liver mitochondria and amine oxidase in plasma. Platelet MAO was found to be similar to the liver enzyme but entirely distinct from the plasma amine oxidase. These investigators consequently suggested using platelet MAO activity as a direct measure of MAO inhibition caused by drugs. Although the inhibitors used had similar effects on both the platelet and liver enzymes, some differences in substrate specificity were noted. Collins and Sandler (1971) found that in contrast to MAO from other tissues, platelet MAO was electrophoretically homogeneous, suggesting that platelet MAO may not necessarily provide an accurate index of MAO activity in the whole organism.

The differences between the platelet enzyme and MAO from other tissues appear to be entirely explained by the finding that human platelets contain only the type B enzyme. Kinetic characteristics and effects of drugs on human platelet MAO are identical to that observed for type B MAO in other tissues (Edwards and Chang 1975). Clorgyline and deprenyl give single sigmoid inhibition curves, with deprenyl being about 200-fold more potent an inhibitor than clorgyline (Edwards and Chang 1975). Tricyclic antidepressants reversibly inhibit platelet MAO with a potency similar to their inhibition of type B MAO in other tissues (Edwards and Burns 1974).

Why human platelets contain only the B form of MAO is not known. It is clear, however, that this is not a general characteristic of platelets, since platelets from the rabbit contain both A and B forms of MAO, whereas rat platelets contain neither form (Edwards and Chang 1975).

Since rabbit platelets contain extremely high concentrations of serotonin, Feldman (1979) considered the possibility that the high activity of type A MAO in rabbit platelets is due to substrate induction. However, this possibility was viewed as unlikely because platelet MAO activity was not increased in patients with chronic hyperse-rotoninemia from metastatic carcinoid tumors (Feldman 1979).

Some Unanswered Questions and Future Strategies

MAO research is progressing in two directions. First, studies at the molecular level are aimed at understanding the chemical nature of the enzyme and the difference between the A and B forms. Other research involves investigations at the physiological level, where the emphasis is on finding the function of the A and B enzymes in the intact organism, and the consequences of alterations in their activity. Both of these approaches may lead to a better understanding of the biological significance of a reduced platelet MAO activity in schizophrenia.

Whether the low platelet MAO activity in schizophrenia is due to a reduced amount of enzyme protein or to an abnormal protein remains to be answered. One approach to this question is to determine the amount of enzyme protein rather than the amount of enzymatic activity. Techniques have been used to titrate A and B active sites in rat liver (Edwards and Pak 1979) could also be used to quantitate the amount of MAO in human platelets.

Another unresolved question is whether there is a reduction in MAO activity in platelets, and perhaps in other tissues, that is sufficient to affect the in vivo metabolism of various biogenic amines, and, if so, which amines are affected. To answer these questions, it will be necessary to quantitatively measure the levels of various biogenic amines and their deaminated metabolites in biological samples, such as plasma or urine.

Toward this end, we have recently developed methodologies that permit us to simultaneously quantitate the concentrations of the neutral, deaminated metabolites of the catecholamines and the trace amines (phenylethanolamine, octopamine, and tyramine) (Edwards, Rizk, and Neil 1979).

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Special Report: Schizophrenia

Single copies of Special Report: Schizophrenia 1976 by Samuel J. Keith et al. are available free of charge from the Center of Studies of Schizophrenia. Multiple copies will also be supplied to requesters who wish to use the report for teaching purposes. The 58-page booklet summarizes recent research in schizophrenia, with special emphasis on work carried out by investigators who have received grant support from the National Institute of Mental Health. The major research areas covered in the report are Diagnosis, Genetics, Biology, Psychophysiology, Psychological Functioning, Family Studies, Studies of Populations at High Risk, Childhood Psychoses, Borderline Conditions, and Treatment. Requests for the report should be addressed to the Center for Studies of Schizophrenia, National Institute of Mental Health, 5600 Fishers Lane, Rm. 10–95, Rockville, MD 20857.