PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 510th Meeting of the Society was held in the May and Baker Research Institute, Dagenham, Essex, on Friday, 20 November 1970, when the following papers were presented:

COLLOQUIUM ON 'MONOAMINE OXIDASE INHIBITORS'

Effects of Monoamine Oxidase Inhibitors on Qualitative Alterations ('Transformation') of the Catalytic Properties of Amine Oxidases

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Treatment with Cu^{2+} in aerobic conditions, oxidized oleic acid or some other oxidizing agents of liver mitochondria from rat (Gorkin & Tatyshenko, 1967) or ox (Gorkin, Akopyan, Veryovkina, Gridneva & Stesina, 1970) reversibly decreases rates of deamination of monoamines and at the same time induces appearance of an ability to catalyse deamination of some nitrogenous compounds including histamine and cadaverine (substrates of diamine oxidases). This phenomenon (operationally termed 'transformation' of monoamine oxidases) has been now observed in similar experiments with highly purified preparations of rat and ox liver mitochondrial monoamine oxidases or tyramine oxidase from *Sarcina lutea*.

We have purified monoamine oxidases of mammalian origin by comparatively simple methods developed in our Institute (Akopyan *et al.* 1970c). Solubilization of monoamine oxidases is achieved by treatment of mitochondrial membranes suspended in distilled water with non-ionic detergents in the presence of benzylamine base, which, as one of the substrates of the monoamine oxidases, protects the enzymes from inactivation in the alkaline medium. Subsequent purification by means of ammonium sulphate fractionation, treatment with alumina gels and hydroxyapatite yields enzyme preparations that, as judged by the values of specific activity and electrophoretic patterns in polyacrylamide gel, do not differ in degree of purification from the highly purified monoamine oxidases described previously, for example, by Nara, Gomes & Yasunobu (1966). Bacterial monoamine oxidase, homogeneous as shown by electrophoresis in polyacrylamide gel, has been prepared by means of a modified procedure by Yamada, Uwajima, Kumagai, Watanabe & Ogata (1967).

All the monoamine oxidases contain about 8 mol of SH group/10⁵ g of protein. Treatment with oxidizing agents (Cu^{2+} , *o*-iodosobenzoate, oxidized oleic acid, ergosterol peroxide) decreases the amount of SH groups titratable by 5,5'-dithiobis-(2-nitrobenzoic acid) down to 2–3 mol/10⁵ g of protein. At the same time the 'oxidized' enzymes acquire the ability to deaminate diamines, polyamines, ω -amino acids and some other nitrogenous compounds (in accordance with the results of our previous experiments carried out with mitochondrial membranes). General patterns of these alterations in catalytic properties are similar in experiments with various amine oxidases, but in details a notable difference is observed. Thus the bacterial tyramine oxidase, which contrary to many other amine oxidases possesses very sharp substrate specificity (Yamada, Kumagai, Uwajima & Ogata, 1967), after treatment with Cu^{2+} or *o*-iodosobenzoate acquires an ability to catalyse the oxidative deamination of L-lysine at an extremely high rate (Yakovlev *et al.* 1970), but histamine is not deaminated by the 'transformed' enzyme. On the other hand highly purified mitochondrial monoamine oxidases from ox or rat liver, deaminating various monoamines besides tyramine and dopamine (3,4-dihydroxyphenethylamine) (the substrates of tyramine oxidase from *Sarcina lutea*), after treatment with oxidized oleic acid or Cu^{2+} readily catalyse the deamination of a large variety of nitrogenous compounds including histamine, lysine, cadaverine, spermine and others. It is possible that treatment with oxidizing agents of individual monoamine oxidases (Gorkin, 1963, 1969; Youdim, Collins & Sandler, 1969) will 'induce' deamination of a restricted number of nitrogenous compounds.

Deamination of histamine by the 'transformed' amine oxidases (i.e. the enzymes pretreated with Cu^{2+} or oxidized oleic acid) obeys an equation of oxidative deamination.

Low concentrations of carbonyl reagents (hydroxylamine, isoniazid, aminoguanidine), in contrast with iproniazid, tranlycypromine and pargyline, inhibit this reaction.

Treatment of the 'transformed' monoamine oxidases with reducing agents (NaBH_4 , H_2S , GSH)

partially restores the initial content of SH groups (up to 6mol/10⁵g of protein) as well as the initial tyramine-deaminating activity, inhibits the ability to deaminate histamine and restores also the inhibitor specificity characteristic for the monoamine oxidases. A similar effect is caused also by a very mild reducing agent, sodium arsenite, which does not reduce disulphide bonds but reduces stabilized residues of sulphenic acid (–SOH) in enzymes to thiol (SH) groups (Parker & Allison, 1969). Our present findings suggest that the chemical basis for 'transformation' of monoamine oxidases may be oxidation of their thiol groups with formation of stabilized sulphenic acid residues rather than disulphide bonds as we have postulated previously (Gorkin, Tatyanyenko & Moskvitina, 1968b).

Treatment of highly purified diamine oxidase from pig kidney (Goryachenkova, Stcherbatyuk & Voronina, 1967) with reducing agents partially inhibits its enzymic activity (substrate putrescine), inducing at the same time an ability to deaminate monoamines (tyramine, tryptamine). This latter reaction is sensitive towards the inhibitory effect of specific monoamine oxidase inhibitors but resistant to the action of carbonyl reagents. Highly purified preparations of the kidney diamine oxidase do not contain free thiol groups. After treatment with H₂S, NaBH₄ or GSH about 6–8mol of SH group/mol of enzyme has been determined by titration with 5,5'-dithiobis-(2-nitrobenzoic acid) or *p*-chloromercuribenzoate. The thiol groups could be reoxidized by means of *o*-iodosobenzoate with simultaneous restoration of initial catalytic properties of the diamine oxidase.

The results of these experiments suggest that, although there is certainly no true interconversion of various amine oxidases, the enzymes possessing quite different prosthetic groups (Tipton, 1968; Nara, Igaue, Gomes & Yasunobu, 1966), both monoamine oxidases and diamine oxidases exhibit significant, previously unrecognized, versatility of their substrate and inhibitor specificity that may be markedly modified by treatment of the enzyme molecules with chemical agents possibly affecting the thiol groups or products of their oxidation.

In radiation injury (Gorkin, Akopyan, Goncharenko & Kudryashov, 1968a), hypervitaminosis D₂ (Akopyan *et al.* 1970a) or after parenteral administration of oxidized oleic acid in doses causing injuries, which closely resemble those produced by whole-body X-ray irradiation (Akopyan, Gorkin, Syomina & Kudryashov, 1970b), we have observed in liver mitochondria a marked decrease in deamination of monoamines and appearance of deamination of histamine and diamines. These findings suggest that the ability of monoamine oxidases to exhibit qualitative alteration ('transformation') of their catalytic properties

may be realized *in vivo*, for example in some experimental pathological conditions accompanied by accumulation of lipid peroxides in tissues.

In agreement with the previously published (Gorkin *et al.* 1968b, 1970) data of our experiments with liver mitochondria we have now established that specific monoamine oxidase inhibitors (pargyline, tranlylcypromine) in conditions ensuring complete inhibition of monoamine oxidase activity *in vitro* prevent alteration of catalytic properties of highly purified monoamine oxidases occurring after treatment of the enzymes with oxidizing agents. Similar effects are caused *in vivo* by pretreatment of rats with iproniazid (150mg/kg) before administration of oxidized oleic acid.

The monoamine oxidase inhibitors may thus interfere, not only with the detoxicating (Blackwell & Marley, 1966) or regulatory (Gorkin & Krivchenkova, 1967, 1970) functions of monoamine oxidases, but also with a striking, but not unprecedented (cf. Colowick, van Eys & Park, 1966), ability of monoamine oxidases to generate in certain conditions *in vitro* and *in vivo* new enzymic activities qualitatively different from those characteristic for the amine oxidases.

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The Reaction of Monoamine Oxidase with Phenethylhydrazine

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The enzyme monoamine oxidase [monoamine-oxygen oxidoreductase (deaminating), EC 1.4.3.4] has been shown to catalyse the oxidative deamination of a wide variety of amines, producing the corresponding aldehyde, ammonia and hydrogen peroxide (Blaschko, Richter & Schlossmann, 1937). The kinetic reaction mechanism has been studied with purified preparations of the enzyme from pig brain mitochondria (Tipton, 1968a) and from ox liver mitochondria (Oi, Shimada, Inamasu & Yasunobu, 1970). In both cases it has been shown that the kinetic reaction pathway is one in which there is no kinetically significant formation of a ternary complex of the enzyme with oxygen and the amine, the reaction taking place by way of a series of binary complexes with a free modified form of the enzyme being produced as an intermediate.

The modified form of the enzyme that is implicated by the kinetic results probably represents a reduced or partially reduced form, and the enzymes prepared from ox liver (Gomes, Igaue, Kloepper & Yasunobu 1969), ox kidney (Erwin & Hellerman, 1967), pig brain (Tipton, 1968b) and rat liver (Sourkes, 1968) have all been shown to contain FAD bound to the enzyme in a ratio of approx. 1 mol of flavin/100 000 g of enzyme protein. In the presence of an amine substrate this flavin has been shown to become reduced. By analogy with the amino acid oxidases (Frieden & Velick, 1957), it may be that the immediate product of amine oxidation is an imine, which then decomposes in the presence of water to form the aldehyde plus ammonia.

A wide variety of hydrazine derivatives have been found to be inhibitors of monoamine oxidase and these compounds have found clinical use as anti-

depressants (for review see Biel, Horita & Drukker, 1964). One of the simplest of these compounds is phenethylhydrazine (also known as phenelzine and Nardil). This compound has been shown to be a powerful monoamine oxidase inhibitor both *in vivo* and *in vitro* (see, e.g., Chessin, Dubnick, Leeson & Scott, 1959) and it has found wide therapeutic use.

Recently Clineschmidt & Horita (1969a,b) have shown that, as well as acting as an inhibitor of monoamine oxidase, phenethylhydrazine also acts as a substrate for the enzyme. These authors did not identify the product of the reaction, which they suggested was phenylacetaldehyde.

Studies on the u.v.-absorption spectrum of the products of the reaction have shown that the spectral properties are not consistent with phenylacetaldehyde being a major reaction product, the spectrum being more consistent with the formation of a hydrazone. If phenylacetaldehyde were formed as a product of the reaction it could rapidly react with the excess of phenethylhydrazine present to form the corresponding phenethylhydrazone. This compound has been synthesized and shown to be a relatively weak reversible inhibitor of monoamine oxidase (Tipton, 1970).

Alternatively the major product of the reaction could be phenylacetaldehyde hydrazone, this compound being formed in the same way as the imine derived from normal amine oxidation, but not being as readily hydrolysed to the corresponding aldehyde. Support for this theory comes from the failure to detect the formation of ammonia during the course of phenethylhydrazine oxidation by monoamine oxidase. The hydrazone generated on the enzyme surface may represent the actual inhibitory species formed from phenethylhydrazine.

Support for the view that oxidation of phenethylhydrazine is intimately linked with monoamine oxidase inhibition comes from studies on the kinetics of the inhibition process. Inhibition by phenethylhydrazine has been shown to be dependent on the presence of oxygen, as was previously reported for the inhibition of monoamine oxidase by *N*-isonicotinoyl-*N'*-isopropylhydrazine (iproniazid) by Davison (1957). The variation of the initial rate of activity loss with oxygen concentration can be interpreted as a Michaelis curve, and the K_m calculated from this is in close agreement with the K_m for oxygen calculated for the direct oxidation of phenethylhydrazine catalysed by monoamine oxidase. Similarly the K_m value calculated from studies of the effect of phenethylhydrazine concentration on the initial rate of inhibition is similar to the K_m value for the oxidation of this compound. The variation of the initial rate of monoamine oxidase inhibition with pH has been found to closely parallel the variation of the initial rate of phenethylhydrazine oxidation with pH. The