Studies on the Active Center of Monoamine Oxidase

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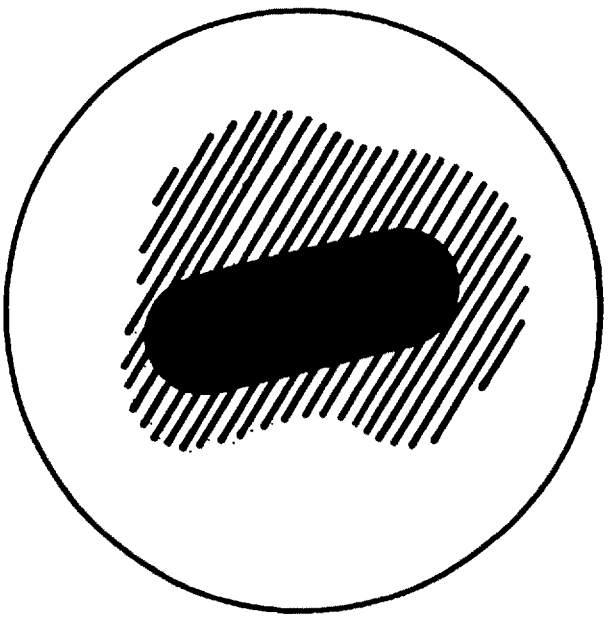
At the occasion of the 500th anniversary of the University of Basel it is altogether fitting that we who went here through the first steps of our academic career should ponder about our debts to this institution. Compared with many universities in other countries, ours is a small one indeed and yet, how many brilliant ideas were born here, ideas strong enough to change man's thinking. Even if we concentrate on biochemistry alone, what an array of famous names arises in our minds, from C. F. SCHÖNBEIN to F. MIESCHER, G. VON BUNGE, and T. REICHSTEIN. The two department heads who opened the door to biochemistry for me were associated for a long time with some of the oldest and most outstanding institutes in this field: before coming to Basel, KARL SPIRO worked for almost 25 years with F. HOFMEISTER in Strassburg, and SIEGFRIED EDLBACHER studied most successfully under A. KOSSEL in Heidelberg. Thus several lines of classical tradition ran within or led to the Department of Physiological Chemistry and we, the younger members of its staff, could in all our pathetic lack of experience not help but feel 'als der Erbe aller Vornehmheit alles vergangenen Geistes und der verpflichtete Erbe', to use the inspired words of F. NIETZSCHE, another teacher at our *alma mater*[1].

It was in S. EDLBACHER's laboratory that I first became acquainted with the enzymology of amino acid and amine metabolism[2]. I also received a helping hand from M. GUGGENHEIM, the author of the classical text on *Die biogenen Amine*. Ever since and with the help of a succession of more than twenty able assistants, I have spent more time and energy on this topic than on any other problem.

Active Centers of Enzymes

Before we approach our subject we have to define the concept of the active center which 'is that special part of the enzyme protein structure which combines with the substrate'[3]. This elegant definition is not always sufficient as the following example will demonstrate: when an aliphatic amine reacts with monoamine oxidase (MO) the amino group as well as the aliphatic chain participate in forming the operative complex with the active center. However, if – in β-position – we introduce a benzene ring, a hydroxy or a second amino group, the new residues also contribute to the binding of these substances to the MO[4]. We must, therefore, differentiate between the primary active center which is always involved in the formation of the enzyme-substrate complex and, around it, a secondary area which only in certain cases establishes bonds with the substrate (Fig.).

The elucidation of the structure of active centers of enzymes is one of the most important, albeit most formidable tasks of modern enzymology. Various ways toward this goal can be chosen. We may determine the amino acid sequence of the active center, which has been marked by a prosthetic group or by substrate or inhibitor molecules[5]. While this procedure often leaves some doubt about the nature and extent of the active center, another approach leads – by definition – directly and unambiguously to the desired enzyme part. It consists of offering a large number of suitably chosen substrates and closely related inhibitors to a given enzyme and deriving, from the kinetic data, affinities and other information. From all these substances we pick out those which fit best into the slot of the active center. Thus gradually a pattern of its structure will emerge. We used mainly this latter procedure in our studies.



Primary and Secondary Area of Active Centers

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Oxidative Deamination

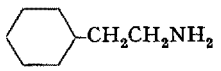
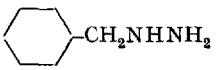
In order to illustrate the concept of active centers we turn to a group of enzymes involved in the oxidative deamination of amines and amino acids. The overall reaction catalyzed by all these enzymes – with some justifiable simplifications – can be summarized by equation I. This apparent similarity

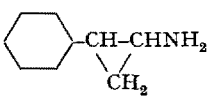
$$\text{I} \quad RR'CHNH_2 + O_2 + H_2O = RR'CO + NH_3 + H_2O_2 \qquad (R' = \text{H or COOH})$$

between the enzymes of this group may not be a mere formal one but could reflect a similarity in action mechanisms. If this were true the actual differences between various amino acid oxidases, monoamine oxidases and diamine oxidases would have to be found in the first steps of the process of the enzyme-substrate interaction. During this phase the substrate molecule is arranged on the active surface in such a manner that the *hydrogen donating* group of the substrate goes into the closest proximity and into the proper spatial relationship to the *hydrogen accepting* residue of the enzyme. It is this sequence of events and the forces directing it which differ from one oxidatively deaminating enzyme to another. – From here on this discussion will be limited to a presentation of MO data.

Substrate Patterns of Monoamine Oxidase

A series of phenylalkylamines were synthesized and tested against various MO preparations[6,7]. Phenylethylamine (Formula II), the compound with the two-membered aliphatic chain, turned out to be the best substrate, both with respect to the affinity (K_M) and reaction rate (V_{max}). When the aromatic ring was left out, the resulting ethylamine was only weakly attacked[8]. Obviously, the amino group, the aliphatic chain, and the benzene ring all are of importance to the formation of the enzyme-substrate complex.

II  III 

IV 

Phenylcycloalkylamines

Since competitive inhibitors have to fulfill fewer conditions for interacting with the enzyme molecule than substrates, it is often relatively simple, with their help, to obtain valuable information about the structure of active centers. As an example the *cis*- and *trans*-phenylcyclopropylamines (see Formula IV) are mentioned[9]. These substances do not qualify as substrates of MO, but they block this enzyme more strongly than any other compound[10–12]. At 10^{-7} M concentration, half of the MO is inhibited ($pI_{50} = 7$). When the benzene ring is left out or when the structure is arranged in a slightly different manner the inactivating power drops tremendously: cyclopropylamine is almost inactive ($pI_{50} = 1{\cdot}5$) and 1-phenyl-cyclopropylamine displays a much smaller efficiency than the 2-phenylcyclopropylamine ($pI_{50} = 5{\cdot}1$).

Inhibition of Monoamine Oxidase by Iproniazid[13]

After the discovery of the remarkable inhibitory power of iproniazid for MO[14–17], one of our principal tasks was to find out whether this compound attacked MO at the same spot as the substrates did. The phenomenon of competitive inhibition[17–18] pointed clearly toward the active center as the primary target of iproniazid. The observations convinced us that iproniazid (V) could become a precious tool for the exploration of MO. Therefore, we took this molecule apart and tested its components and related structures on MO[6,19,20]. After having studied a great number of substances it became obvious that the effect of iproniazid on MO was due to the isopropylhydrazine residue (see bold printed part of the iproniazid molecule V) in much the same way as in the classical work of TRÉFOUEL, BOVET, and NITTI, the sulfanilamide residue was recognized as the essential part of Prontosil.

V 

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Phenylalkylhydrazines

Experiments carried out with iproniazid induced us to test alkylhydrazines and phenylalkylhydrazines which turned out to be excellent MO-blocking agents. Some data obtained with a series of phenyl-*n*-alkylhydrazines ($C_6H_5(CH_2)_nNHNH_2$, $n = 0$ to 3), may serve for illustration. In this series benzylhydrazine ($n = 1$, see Formula III) was the most effective agent, closely followed by phenylethylhydrazine ($n = 2$)[6,7]. In addition to benzylhydrazine, some analogous substances such as 2-thiophene-methylhydrazine and 3-pyridine-methylhydrazine inhibited MO more strongly than most of the hydrazine derivatives[7].

Homologous Enzymes

When data on substrate and inhibitor patterns are discussed one has to keep in mind that they are valid only for a given species or organ because the enzymic response to various substances changes with the source from which the enzyme was prepared. This is certainly true for MO[8,21–23]. One example has to suffice: rabbit liver MO is much less sensitive ($pI_{50} = 5{\cdot}7$) toward the phenylcyclopropylamines than beef liver MO[10]. The difference cannot be accounted for by differences in the accessibility of MO in the two kinds of mitochondria since solubilized rabbit liver MO displayed the same behaviour as that of whole mitochondria. Following the terminology of comparative anatomy it has been suggested that a group of closely related, but not identical enzymes, be called homologous[24,25]. The chemical differences between homologous enzymes may pertain to amino acids away from the active center, as seems the case for sheep and beef pancreas ribonuclease[26], or within the active center, as some data mentioned above and related informations about MO suggest.

Active Center of Monoamine Oxidase

All three outstanding agents – phenylethylamine II, benzylhydrazine III, and phenylcyclopropylamine IV – display the same backbone: a benzene ring, a two-membered side chain, and an amino group. When the benzene ring is left out, the substrate or inhibitor activity drops sharply or disappears almost entirely. When we remember how closely related CH_2- and NH-residues are – as expressed by GRIMM's theorem of the hydride shift (Hydridverschiebungssatz) and demonstrated with many striking examples by ERLENMEYER[27] – the similarity becomes even greater. One can, therefore, hardly avoid the idea that all classes of compounds considered here, amines as well as pseudoamines, act in a similar fashion with MO. Furthermore, the active center of this enzyme may contain a structure complementary to the amines and hence may be made up by an aromatic nucleus and a two-membered side chain[28]. Between the related structures of the substrate of inhibitor molecules and of the active center hydrophobic forces may come into play and help to adjust the smaller molecules to the enzyme surface.

Our results with α-substituted aliphatic amines and hydrazines can be interpreted by the assumption that *one* α-hydrogen is essential for establishing the enzyme-substrate complex or enzyme-inhibitor complex, while the *second* α-hydrogen—if one is available—participates in the process of dehydrogenation. One of several possibilities consists of assigning to one part of the active center the following structure, $-X\text{–}\ddot{Y}-$, in which X serves as an acceptor for the α-carbon, and $\ddot{Y}$ for a proton (derived from an α-hydrogen)[10].

Many other properties of the active center of amine oxidases have been studied with the help of specifically selected substrates and inhibitors in this and other laboratories, but we must limit ourselves here to two conclusions: the active center of these enzymes seems to extend over several *Angstrom* units[29] and its structure appears to be asymmetrical[23,30–32] possibly because it is built up in part by L-amino acids[30].

In vivo Experiments

Shortly after noticing the strong blocking power of iproniazid on MO *in vitro* we observed the equally effective *in vivo* inhibition of brain and liver MO by this drug [15–17]. It took several days before the enzymic activity recovered[17,33], long after all free iproniazid had disappeared from the animal[34]. Similar results were obtained with various species[35] and related inhibitors, including phenylcyclopropylamines[10].

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MO-Inhibitors as Tools in Metabolic, Pharmacological, and Clinical Research

One may ask whether MO and in particular its active center are more than a plaything for the enzymologist. With the following remarks—by necessity extremely brief[36]—it is attempted to answer this question.

Since iproniazid and similarly effective compounds block MO substantially *in vivo* they possibly could alter the metabolism of those biogenic amines which are substrates of MO. This has been shown to be true for the first time by SCHAYER at Northwestern University who was able to increase the urinary excretion of labelled tyramine in iproniazid pretreated rats[37]. Others demonstrated that, in brain, heart, urine, and other materials, the level of various amines such as serotonin and catecholamines goes up after blocking MO[35, 38, 39]. Obviously, these highly active compounds are protected from being destroyed by MO in much the same way as LOEWI prevented acetylcholine by means of eserine[40] from being hydrolyzed by cholinesterase.

The enzymic and metabolic studies described here have thrown an entirely new light on the pharmacology of iproniazid and related drugs which may primarily act by modifying the metabolism of certain biogenic amines. The first experiments confirming this idea were carried out in the Departments of Biochemistry, of Pharmacology—C. A. DRAGSTEDT, E. C. GRIESEMER, J. A. WELLS—and of Medicine—S. M. FEINBERG, J. REBHUN—of Northwestern University. We showed that the effect of tyramine on the nictitating membrane of the cat[41] and on the body temperature of the guinea pig [42] was strongly potentiated by iproniazid. Since then a large number of investigations have been performed first by BRODIE and then by other outstanding pharmacologists. Among other things they contributed to a better understanding of the pharmacology of reserpine[35, 36, 39].

By now an impressively large body of informations has been accumulated, compatible with the concept that the active center of MO is one of the targets—if not the main one—of iproniazid. This drug was first introduced as an antituberculosum but was subsequently rejected because of some undesirable effects[43]. In two independent experiments the influence of iproniazid on psychotic patient was investigated, this time based on the MO-blocking concept. One study was conducted at the Rockland State Hospital, Orangeburg, New York, by KLINE *et al.*[44] and the other at the Veterans Administration Hospital in Hines (Illinois)—BERNSOHN, INSKIP, LAUER—and at Northwestern University[44]. Today probably more than a million patients suffering of psychoses and other diseases have been treated by 'psychic energizers'.

It is beyond the scope of this paper to evaluate the role of MO in the many therapeutic applications of the so-called MO-inhibitors or to discuss the data presented at national and international meetings which have been devoted in part or *in toto* to the monoamine oxidase inhibitors[36].

Thus a large tree has grown in the rich soil of the 'New World' from small seeds sown at the University of Basel and the concepts and methods of theoretical enzymology have become handy tools for many segments of biology in general and clinical medicine in particular[45].

Zusammenfassung

Zunächst werden primäre und sekundäre Anteile der aktiven Enzymzentren und oxydative Desaminierung definiert. Es wird dann ein allgemeines Verfahren geschildert um durch die Exploration des Substrat- und Inhibitorbereichs das aktive Zentrum der Monoamin-oxydase (MO) zu untersuchen. Phenylalkylamine, Phenylcyclopropylamine und Phenylalkylhydrazine erreichen dann ihre maximale Wirkung, wenn sie aus einem aromatischen Ring und aus einer zweigliedrigen Seitenkette bestehen. Schliesslich wird der Einfluss dieser Studien, die zum Teil ihren Ausgang von der Analyse des Iproniazideffekts nahmen, auf die Erforschung des Stoffwechsels, der Pharmakologie und der Klinik biogener Amine angedeutet.

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CONGRESSUS

Sweden

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