INHIBITION OF GLUTAMIC-PYRUVIC TRANSAMINASE BY DL-CYCLOSERINE AND OTHER COMPOUNDS

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In a previous study [1] we showed that D,L-cycloserine at concentrations as low as $2 \cdot 10^{-5}$ M significantly inhibits glutamic-pyruvic transaminase activity in rat liver homogenates. Acyclic precursors of cycloserine—ethyl ester of β-aminohydroxyalanine (KX-9) and β-aminohydroxyalanine (KX-14)—proved to be effective inhibitors as well.

According to the hypothesis of N. K. Kochetkov et al. [2], the free amino group of cycloserine forms with the aldehyde group of phosphopyridoxal Schiff bases which, under mild conditions, can be converted into stable complexes and thus block the enzyme. From this point of view, a necessary prerequisite for blocking of the enzyme is the presence in the inhibitor molecule of a free amino group and a molecular configuration which ensures conversion of the azomethene compound to stable form.

To clarify these matters, we investigated the effectiveness of a number of cycloserine derivatives, and also of certain other compounds *.

Information on the structure of compounds investigated is presented in the table.

METHOD

Experimental conditions and methods were the same as those employed in the preceding study [1]. Glutamic-pyruvic transaminase activity in liver homogenates was determined according to increase in alanine and decrease in pyruvic and glutamic acids. In those cases where inhibitor concentration was below 10^{-3} M, i. e., was 20 times lower than substrate concentration, we disregarded the fact that some of the inhibitors can bind pyruvic acid, or that some are not separated from alanine or glutamic acid during chromatography.

In experiments with higher concentrations, additional control samples were run in which one of the substrates was omitted, and percent inhibition was calculated taking into account the values observed for these samples. Many of the inhibitors bound the carbonyl group of pyruvic acid, thereby removing it from the reaction and being themselves "neutralized". In those cases, we carried out a preliminary incubation of homogenate with inhibitor in order that the inhibitor might act on the transaminase before addition of substrate.

RESULTS

Average figures for 5-6 experiments are presented in the table. They reveal that the acyclic compounds investigated are able to inhibit glutamic-pyruvic transaminase in greater or less degree. In the preceding study [1] we believed it justifiable to assume that the strong effect of KX-9, equal to that of cycloserine, is perhaps related to

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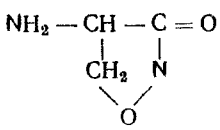
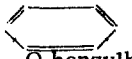
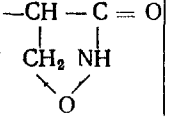
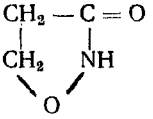
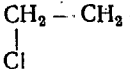
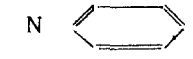
Percent Inhibition of Glutamic-Pyruvic Transaminase Activity

Arbitrary designation	Compound	Concentration (moles)								
		10^{-4}	10^{-3}		$2\cdot10^{-3}$		10^{-2}		$2\cdot10^{-2}$	
		-p.	-p.	+p.	-p.	+p.	-p.	+p.	-p.	+p.
	Cycloserine 	100	—	—	—	—	—	—	—	—
KX-9	$NH_2O-CH_2-CHNH_2-COOC_2H_5 \cdot 2HCl$ Ethyl ester of β-aminohydroxyalanine (dihydrochloride)	100	—	—	—	—	—	—	—	—
KX-14	$NH_2O-CH_2-CHNH_2-COOH \cdot HCl \cdot H_2O$ β-aminohydroxyalanine (monohydrochloride)	40	75	—	—	—	—	—	—	—
KX-25	$NH_2O-CH_2-CH_2-CH_2-COOC_2H_5 \cdot HCl$ Ethyl ester of γ-aminohydroxybutyric acid (hydrochloride)	—	60	—	100	—	—	—	—	—
KX-24	$NH_2O-CH_2-CH_2-CH_2-COOH$ γ-aminohydroxybutyric acid	—	30	—	100	—	—	—	—	—
O-BHA*	 $-CH_2-ONH_2 \cdot HCl$ O-benzylhydroxylamine (hydrochloride)	—	—	—	36	—	—	100	—	—
KX-10	 N-methylcycloserine	—	—	35	0	—	80	90	—	—
AH-2	 Isoxazolidone-3	—	20	23	—	—	60	—	—	80
AH-1*	$CH_3-CHNH_2-CO-NHOH$ Hydroxamic acid of α-alanine	—	0	—	—	—	—	—	—	70
AH-3	 β-chlorpropionhydroxamic acid	—	—	40	—	—	—	—	—	92
Isoniazide*	Hydrazide of isonicotinic acid 	—	—		—	—	—	20	—	46

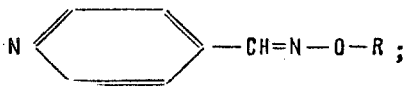
Note: –p.–without preincubation of enzyme and inhibitor; + p. – with preincubation.

* Strongly binds pyruvic acid.

its ability to cyclize relatively easily with formation of cycloserine. It follows from the data of M. A. Breger obtained in the Institute of Pharmacology and Chemotherapy, however, that this compound has a considerably weaker effect on the growth of Mycobacterium tuberculosis bovin 8 than does cycloserine; this casts doubt on the possibility of cyclization of KX-9 to cycloserine.

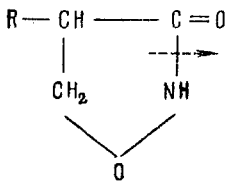
It was also considered possible that pH affects cyclization of this compound. In special experiments it was found, however, that percent inhibition is practically the same at pH 6.5 and pH 7.5. These observations, taken in conjunction with the fact that other acyclic compounds (KX-14, KX-24, KX-25) are also effective inhibitors of transamination, gave reason to believe that KX-9 as such is able to inhibit glutamic-pyruvic transaminase.

Of great interest are the findings that compounds lacking an amino group, but containing a hydroxyamino group, are likewise effective inhibitors (KX-24, KX-25, and O-BHA). If it is granted that the hydroxyamino group of these compounds reacts with the aldehyde group of phosphopyridoxal, obviously a compound of the following type must be formed:



this must be fairly stable in order to block the enzyme.

KX-10 and AH-2, compounds which contain neither a free amino group nor a hydroxyamino group, also possess definite inhibitory activity. If the activity of these compounds is explained in terms of Kochetkov's hypothesis [2], it must be assumed that the ring is ruptured during interaction with the enzyme to free some grouping capable of combining with the aldehyde group of phosphopyridoxal. It is theoretically possible that the ring of isoxazolidone-3 (AH-2), as well as that of N-methylcycloserine (KX-10), is ruptured hydrolytically between the amino nitrogen and the carbonyl carbon:



A rupture of this type must certainly yield a hydroxyamino compound which, as our data show, is an effective inhibitor of glutamic-pyruvic transaminase. Against this assmuption, however, is the fact that no binding of pyruvic acid could be detected when AH-2 was incubated together with enzyme and pyruvic acid, a reaction which must have occurred if a hydroxyamino group were actually formed.

It is of interest that in two cases (see KX-9 and KX-14, and also KX-25 and KX-24) the ethyl esters of acids proved more effective than the acids themselves. It is at present difficult to explain this in any way.

In connection with the considerations of Metzler et al. [7] concerning the role of heavy metal ions in transamination, the observation of N. K. Kochetkov et al. [2],that cycloserine and also a number of its azomethene derivatives are able to form complexes with heavy metal ions,is of interest. This is also true of isoniazide. Cymerman-Craig et al. [4] found a parallelism between the capacity of a number of substituted isoniazides to form such complexes and their capacity to suppress growth of tuberculosis bacilli. It has been suggested that complex formation may prevent normal transamination. This is belied by the finding, however, that EDTA does not inhibit enzymatic processes involving phosphopyridoxal [3,8]. It should be noted, though, that in non-enzymatic transamination EDTA disrupted the metal-phosphopyridoxal-glycine complex [6]. Matsuo believes that the absence of an EDTA effect in enzymatic reactions may be explained by the presence of an apoenzyme which stabilizes the complex of metal with Schiff bases.

In view of these considerations, it seemed to us of interest to determine the effect on glutamic-pyruvic transaminase of hydroxamic acids, which are capable of complex formation. We tested two hydroxamic acids, of which AH-1 may be considered a geometric analogue of cycloserine, and AH-3 a precursor of isoxazolidone. The table shows that both compounds were effective inhibitors, AH-3, with no free amino group, being even more

effective than AH-1, which possesses this group. Thus, all the compounds tested, with the exception of KX-9, proved considerably less effective than cycloserine, but more effective than the extensively used inhibitor of transamination, isoniazide.

From their study of possible mechanisms involved in isoniazide inhibition of transamination, Hicks and Cymerman-Craig [5] concluded, nevertheless, that in all probability the hydrazide group of isoniazide interacts with the aldehyde group of phosphopyridoxal.

Our investigations show that compounds lacking groupings capable of interacting with the aldehyde group of phosphopyridoxal are even more effective inhibitors than isoniazide. It is difficult to believe that the mechanism of inhibition is in principle the same for so strong an inhibitor as cycloserine and so weak a one as isoniazide, while it is different for inhibitors of intermediate effectiveness. Such another mechanism must be postulated for hydroxamic acids, in any case, if it is assumed that the rings of N-methylcycloserine and isoxazolidone-3 can be ruptured. The existence of complex-forming properties in the majority of compounds investigated provides some basis for the supposition that such properties determine the effect of these compounds on glutamic-pyruvic transaminase.

These findings on the effect of various compounds, which may or may not be able to form Schiff bases, indicate that transaminase inhibition may occur by some means other than that involving interference with the reaction of phosphopyridoxal with amino acid substrate.

SUMMARY

The author studied changes in glutamic-pyruvic transaminase activity of liver homogenates under the effect of various compounds capable and incapable of forming Schiff bases with phosphopyridoxal.

The following compounds proved effective in inhibiting the glutamic-pyruvic transaminase activity in the homogenates of rat liver: 1) acyclic-containing both amino and hydroxyamino groups, or hydroxyamino group alone (β-aminohydroxyalanine and γ-aminohydroxybutyric acid and their ethyl esters); 2) cyclic-differing from cycloserine by the absence of a free amino group (N-methylcycloserine and isoxazolidone-3); 3) hydroxamic acids (hydroxamic acid of α-alanine and β-chlorpropionhydroxamic acid). All these compounds, except for the ethyl ester of β-aminohydroxyalanine, the efficacy of which equals that of cycloserine, are less effective than cycloserine; their efficacy, however, is greater than that of isoniazide. Possible mechanisms of action of the above inhibitors are discussed.

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