BBA 63322

Inhibition of invertases by pyridoxal and its analogues

During a search for a specific inhibitor of potato invertase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26), I discovered that pyridoxal strongly inhibits this enzyme. The availability of analogues of pyridoxal prompted a study to determine the effect of altering the structure of pyridoxal on the inhibition of potato invertase and also invertases from Neurospora and yeast. Aniline, a well-known inhibitor of invertases, was included in this study for comparative purposes.

Potato invertase was partially purified from cold-stored tubers according to the procedure described earlier[1]. The specific activity of the preparation was 140 units per mg protein. Neurospora invertase was generously provided by Dr. R. L. METZENBERG, University of Wisconsin, Madison. Yeast invertase and the pyridoxal analogues were purchased from the Sigma Chemical Co., St. Louis, Mo. The inhibitors were dissolved in 0.08 M acetate buffer (pH 4.5) with adjustment of the pH to 4.5, if necessary, just before use.

The incubation mixture for the invertase assay contained 0.4 mmole of sodium acetate buffer (pH 4.5), an appropriate amount of substrate, and 20 units of invertase[1] in a total volume of 5 ml. After a 1-h incubation at 37°, the reactions were stopped by adding 5 ml of 0.5 M dibasic phosphate and heating in a boiling-water bath for 4 min. The reducing sugars formed in the enzymic reaction were measured by the arsenomolybdate method[2]. Blanks consisting of the incubation mixture less the substrate were run to correct for the effects of some inhibitors on the analytical method.

A summary of the results is presented in Table I. The inhibitions by all the inhibitors appeared to be reversible and non-competitive. The inhibition constants were determined by the graphical method of DIXON[3]. While keeping the substrate concentration constant, the velocity was determined with a series of inhibitor concentrations. Plotting the reciprocal velocity against inhibitor concentration yielded a straight line. The straight lines obtained for a number of different substrate concentrations intersected on the base-line and this intersection gave $-K_i$. Inhibition constants greater

TABLE I

INHIBITION CONSTANTS FOR THE INHIBITION OF POTATO, YEAST AND NEUROSPORA INVERTASES BY PYRIDOXAL ANALOGUES AND ANILLINE

Inhibitor	K_i *(mM)*					
	Potato invertase		*Yeast invertase*		*Neurospora invertase*	
	Sucrose	*Raffinose*	*Sucrose*	*Raffinose*	*Sucrose*	*Raffinose*
Pyridoxal	4	23	29	110	21	36
Pyridoxine	18	48	27	35	24	21
Deoxypyridoxine	17	20	15	16	30	18
Pyridoxal phosphate	6	16	56	56	*	*
Pyridoxamine	60	140	*	*	*	*
Aniline	110	*	1.7	6	1.5	5

* K_i values greater than 150 mM.

than 150 mM were omitted because of difficulties in working with high concentrations of many of the reagents.

Of the reagents tested, aniline is the most potent inhibitor of yeast and Neurospora invertases but the weakest inhibitor of potato invertase. The inhibitions of yeast[4] and Neurospora[5] invertases by aniline have been studied in detail. Studies with substituted anilines on the inhibition of yeast invertase have disclosed that, in general, substituents which increase the basicity increase inhibition but steric effects are also important[4]. Aniline inhibition of Neurospora invertase has been interpreted as a strong combination of aniline with the enzyme–fructose complex[5]. This mechanism offers an explanation for an aniline inhibition constant of higher molarity for the substrate raffinose than for sucrose[5].

Most of the analogues of pyridoxal are more effective inhibitors of potato invertase than of yeast and Neurospora invertases. The differences between potato invertase and the other two invertases are greatest for pyridoxal and pyridoxal phosphate, which are the most effective inhibitors of potato invertase. In contrast, most inhibition constants for each of pyridoxine and deoxypyridoxine do not differ by more than a factor of two for all three enzymes. Pyridoxine and deoxypyridoxine are also nearly equally effective for each enzyme. Pyridoxamine is the least effective inhibitor for each of the three invertases but is more effective for potato invertase than for the other two invertases. In most cases, inhibition constants are lower for the substrate sucrose than for raffinose. However, the inhibition constants for both substrates are similar for a number of the inhibitors. For deoxypyridoxine inhibition of Neurospora invertase, the inhibition constant is lower for raffinose than for sucrose.

It is not certain if pyridoxal analogues and aniline function as invertase inhibitors by identical mechanisms. However, it may be assumed that pyridoxal analogues are further examples of aromatic amines which inhibit invertase. Undoubtedly the pyridinium group is involved in binding at the inhibition site because it is the only ionically charged group in pyridoxal, pyridoxine, and deoxypyridoxine. If the basicity of the pyridinium group were the only important factor, there would not be much effect on inhibition by interchanging the groups $-CHO$, $-CH_2OH$, and $-CH_3$ on carbon atom 4. This is essentially the case for yeast and Neurospora invertases which are equally inhibited by pyridoxal, pyridoxine, and deoxypyridoxine. Because pyridoxal is distinctly more effective than pyridoxine and deoxypyridoxine for potato invertase, implies that steric or secondary binding effects are also important, at least for this enzyme. A $-CHO$ group on carbon atom 4 is the most favorable and a $-CH_2NH_2$ group is the least favorable for binding at the inhibition site. Unexpectedly, introduction of a phosphate group into pyridoxal does not markedly decrease inhibition of potato invertase as it does for yeast and especially Neurospora invertase.

The invertases from potatoes, yeast, and Neurospora are known to differ in several ways. The Michaelis constants for sucrose hydrolysis have been reported to be 3 mM for Neurospora invertase[5], 4 mM for potato invertase[1] and 26 mM for yeast invertase[4]. Potato invertase is the only one of the three invertases which is inhibited by potato invertase inhibitor[1]. Because this macromolecular inhibitor reacts irreversibly with the free enzyme[1], the potato invertase differs from the other two enzymes in whatever feature is required for combination with the inhibitor. Whereas the studies with potato invertase inhibitor indicate radical differences, the results obtained in the present study suggest relative differences. The enzymes are all inhibited by aniline

and analogues of pyridoxal. However, because the order of effectiveness is different for each invertase, the inhibition sites are similar but not identical.

*Red River Valley Potato Processing Laboratory**, RUSSELL PRESSEY
East Grand Forks, Minn. 56721 (U.S.A.)

1 R. PRESSEY, *Plant Physiol.*, 42 (1967) 1780.
2 N. NELSON, *J. Biol. Chem.*, 153 (1944) 375.
3 M. DIXON, *Biochem. J.*, 55 (1953) 170.
4 K. MYRBÄCK, in P. D. BOYER, H. LARDY AND K. MYRBÄCK, *The Enzymes*, 2nd ed., Vol. 4, Academic Press, New York, 1960, Chapter 22.
5 J. R. TREVITHICK AND R. L. METZENBERG, *Arch. Biochem. Biophys.*, 107 (1964) 260.

Received March 4th, 1968

* A laboratory cooperatively operated by the Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture; Minnesota Agricultural Experiment Station; North Dakota Agricultural Experiment Station; and the Red River Valley Potato Growers' Association.