0.72x + 8.35 and correlation coefficient 
\( r = 0.999 \). This means that there is absolutely no indication of a different affinity of inhibitor to substrate, or of a greater degree of dissociation of the enzyme-inhibitor complex. In fact, the gradual increase in the reaction rate after 10 min, as observed by Huang and Tietz in their assay in the presence of inhibitor, might be a reflection of the lower inhibitor concentration they used. Nevertheless, the introduction of a linear standard curve by Huang and Tietz certainly adds to the attractiveness of the method of isoamylase determination by selective inhibition.

References


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Drs. Huang and Tietz respond:

To the Editor:

We are pleased that F. J. Hoek has found essential agreement between the linear standard curve presented in our article and that obtained by O'Donnell et al., by using a different presentation of O'Donnell's data. We take this as a confirmation of the validity of the experiments conducted in the respective laboratories.

We speculated in our publication on the possible reasons for the nonlinear standard curve by O'Donnell, but we expended little effort on this aspect, because it did not affect the validity or usefulness of our data. The significantly lower amount of inhibitor used in our version of the method continues to be a major financial advantage, and the shorter incubation is a real convenience.

We hope that Hoek's Letter will help to promote the use of isoamylase determinations by selective inhibition.

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Inactivation of Alkaline Phosphatase

To the Editor:

Rej et al. (1) reported that a trace amount of 2-amino-3-aza-2,5-trimethylhexanol (AATMH), present as a contaminant in the 2-amino-2-methyl-1-propanol used as buffer, inactivates alkaline phosphatase (EC 3.1.3.1) from several sources. A specific and effective inactivator of alkaline phosphatase would be very useful in eliminating this activity when it interferes with other assays (2) or after alkaline phosphatase-catalyzed digestion of nucleic acids before labeling with \( ^{32} \)P (3). We have therefore examined AATMH for its usefulness as an inactivator of alkaline phosphatase under several conditions.

At a concentration of 10 mmol/L, AATMH (Aldrich Chemical Co.) in 100 mmol/L ethanolamine hydrochloride buffer, pH 8 to 10.5, we saw no inactivation of human placental alkaline phosphatase (Sigma) over a period of 30 min at 30°C. However, an instantaneous pH-dependent, time-independent inhibition was observed. This inhibition averaged 22% at pH 8.0, 8% at pH 9.25, and 43% at pH 10.5. When these assays were repeated in the presence of MgCl\(_2\) (1 mmol/L), 15% inhibition was found at pH 8.0 and 9.25, and 24% at pH 10.5. In addition, a slow inactivation (half-life on the order of 20 min) was observed at pH 9.25 and 10.5 in the presence of MgCl\(_2\). We have never been able to completely inactivate with AATMH, but EDTA gave instantaneous and complete inhibition. In the presence of 10 μmol of Zn\(^{2+}\) per liter, AATMH did not inactivate the enzyme, in agreement with the observations reported by Rej et al. (1). The effects of Mg\(^{2+}\), Zn\(^{2+}\), and EDTA seem to support the suggestion (1) that AATMH acts by interfering with the enzyme's Zn\(^{2+}\) requirements.

We conclude that inactivation by AATMH is too dependent on reaction conditions and on the source of the enzyme (we observed stimulation of the activity of the calf intestinal enzyme under some conditions) to be useful as a specific inactivator of alkaline phosphatases.

References


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ß2-Microglobulin in Urine: Not Suitable for Assessing Renal Tubular Function

To the Editor:

I was interested in the communication by Bernard and Lauwerys (Clin Chem 27:1781–1782, 1981) and the latter articles by Davey and Gosling (1) and Bernard et al. (2) showing that the activity of ß2-microglobulin (ß2-m) in urine declines at a pH below 6.0. Scharfijn et al. (3) indicated that patients in whom ß2-m is to be measured must be given sufficient bicarbonate to ensure that they produce alkaline urine declines at a pH below 6.0. Scharfijn et al. (3) indicated that patients in whom ß2-m is to be measured must be given sufficient bicarbonate to ensure that they produce alkaline urine (pH 6.0), so that ß2-m will not be degraded in the bladder—but this is not practicable for most clinical purposes.

Figure 1 shows results of a pH study of 177 random (untimed) urines sub-