A Cyanide-Aldehyde Complex Inhibits Bacterial Luciferase

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Cyanide at high (millimolar) concentrations inhibited in the in vitro Vibrio harveyi luciferase reaction. Cyanide reacted with free aldehyde to form an inhibitor. Inhibitor formation was accelerated by alkaline conditions and bovine serum albumin.

It has long been known that, under some conditions, cyanide at low concentrations (10^{-3} to 10^{-6} M) can increase the intensity of bacterial bioluminescence (3). This has been attributed to its inhibition of cytochromes in the electron transport pathway, resulting in an increase in the cellular levels of reduced NAD and reduced flavin mononucleotide (FMNH_{2}), the latter being a substrate for luciferase in the light-emitting reaction (6). However, it has recently been shown that in vivo luminescence in Vibrio harveyi and V. fischeri is inhibited at higher (millimolar) cyanide concentrations (7). Cyanide inhibition of bioluminescence could be reversed by the addition of fatty aldehyde, the cosubstrate of the luciferase reaction.

The present study was undertaken to investigate the nature of cyanide inhibition of luciferase in vitro. V. harveyi luciferase was purified by ammonium sulfate fractionation, DEAE, and affinity chromatography (1, 4). Luciferase was assayed by the standard (nonturnover) assay in 0.05 M Na^{+}/K^{+} phosphate buffer, pH 7.0, containing 0.2% bovine serum albumin (BSA) by injection of 1 ml of 0.05 mM catalytically (H_{2} and platinum asbestos) reduced FMN (in the same buffer lacking BSA) into 1 ml of standard assay buffer containing a dilution of the enzyme in microliter amounts with 5 μl of a 5 mM solution of decanal in ethanol. The peak height (I_{0}) was used to calculate the enzyme activity (4). As shown below, BSA enhanced the inhibitory effect of cyanide; consequently, in some experiments, no or lesser amounts of BSA were used. Cyanide solutions (1 M, KCN) were prepared fresh daily and diluted for use as required.

In the standard luciferase assay, cyanide had little or no inhibitory effect when added a few seconds (<5 s) before injection of FMNH_{2}. Moreover, the addition of cyanide after the onset of the reaction also had no effect on the kinetics of the decay thereafter. However, when aldehyde was allowed to incubate with 10 mM cyanide in buffer prior to the addition of luciferase and FMNH_{2}, there was a significant inhibition (decrease in I_{0}); after 1 min, 50% inhibition was apparent (Fig. 1). In the presence of BSA, the inhibition was >99%.

When the reciprocal of percent inhibition was plotted versus 1/[BSA] for various concentrations of BSA, the plot yielded an apparent K_{m} for BSA in this reaction of 2.4 \times 10^{-6} M or 0.154 mg of BSA per ml. The effect of cyanide was also shown to be concentration dependent, and a plot of 1/percent inhibition versus 1/[cyanide, millimolar] gave an apparent K_{m} for the formation of the cyanide-aldehyde inhibitor of 0.033 mM cyanide in the presence of BSA.

In all of these experiments, the enzyme was added just prior to the injection of reduced flavin. It was concluded that the inhibitory effect of cyanide is due to its reaction with aldehyde to form an inhibitor rather than a direct effect of cyanide on luciferase itself. Incubation of luciferase with 10 mM cyanide for 1 min resulted in little or no inhibition when aldehyde was added a few seconds prior to injection of FMNH_{2}. Moreover, the presence of luciferase during the reaction of decanal with aldehyde did not alter the extent of the inhibition. Therefore, luciferase does not enhance the production of inhibitor by BSA does.

The inhibitory action of the postulated cyanide-decanal inhibitor, like other aldehyde analogs, is attributed to reversible binding to the enzyme flavin hydroperoxide (2, 5, 8). This results in a decrease of I_{0} as well as of the apparent first-order decay constant, k, and the quantum yield (Table 1). The kinetic effect of inhibitors formed with cyanide and other aldehydes (C_{6}, C_{12}), whose reaction with luciferase is kinetically slow, was barely distinguishable from the decay of the uninhibited reaction: therefore, this study reports only results with decanal, the C_{10} aldehyde.

The formation of the intermediate II-inhibitor complex, and its reversible nature, can be shown by the secondary addition of excess aldehyde to the inhibited reaction during its decay (2, 8). Injections of aldehyde at different times after the initiation of the reaction recovered intermediate II from its cyanide-inhibitor complex, producing a flash with a fast decay rate (k = 0.16 s^{-1}) but not as fast as that of the
reaction in the absence of inhibitor \( (k = 0.385 \text{ s}^{-1}) \). The peak intensity values produced from the secondary additions of aldehyde declined in parallel with the decay of the inhibited reaction \( (k = 0.045 \text{ s}^{-1}) \).

The compound first formed by the reaction of cyanide with aldehyde might be the cyanohydrin (Fig. 2), which could be the inhibitor. However, the cyanohydrin would be expected to break down in water to form either the alpha hydroxy acid or amide \((9, 10)\), either or both of which might also act as inhibitors. Cyanohydrin formation is favored by alkaline conditions \((9, 10)\), which also favored the formation of the inhibitor \( (\text{Fig. 3A}) \). In the presence of BSA, the inhibitor was also formed faster in alkaline conditions, but inhibitor was formed even at pH 6.0 \( (\text{Fig. 3B}) \), suggesting that BSA may provide some sort of microenvironment that facilitated the nucleophilic attack of cyanide on aldehyde to form the cyanohydrin.

It is postulated that cyanide inhibition of in vivo bioluminescence \((7)\) involves a similar mechanism: after entering the bacterium, cyanide combines with the natural aldehyde, thereby forming the cyanohydrin, which could possibly be converted by water to the corresponding alpha hydroxy acid or amide. One or more of these could bind luciferase intermediate II, thereby inhibiting bioluminescence and trapping intermediate II in an inhibitor complex. That such a reversible complex is formed is supported by the flash of light obtained upon the exogenous addition of excess aldehyde to bacteria inhibited by cyanide \((7)\).

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