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## Effect of Diisopropylfluorophosphate on Sulfhydryl Proteases

*Abstract.* Diisopropylfluorophosphate inhibited all the sulfhydryl proteases studied in our tests. This inhibition was most pronounced at *p*H 6.0. By first blocking the sulfhydryl group with *p*-chloromercuribenzoate, inhibition could be prevented. Neither cysteine nor choline gave appreciable reactivation of diisopropylfluorophosphate-inhibited bromelain.

Although the organic phosphate nerve gases are well established as specific inhibitors of certain esterases, trypsin and chymotrypsin, activated plasmin and phosphoglucomutase (*1*), no reports of specific inhibition of the sulfhydryl enzymes have been made (*2*).

In a comparative study of plant proteases we found that moderate concentrations of diisopropylfluorophosphate inhibited many preparations of bromelain (*3*), papain, and ficin. With commercial bromelain, $4 \times 10^{-4}M$ diisopropylfluorophosphate inhibited 50 percent of the protease activity within 2 hours at room temperature. Some of our fractionated bromelain preparations, on the other hand, showed only slight inhibition at high concentrations of reagent.

The effectiveness of diisopropylfluorophosphate as an inhibitor depended not only on the type of enzyme and the degree of purity of the enzyme but also on the *p*H of the solution (Fig. 1). Bromelain and papain, which presumably have similar active sites, showed an entirely different behavior at all *p*H values below *p*H 5.0. On the other hand, Rhozyme P-11, a fungal protease which does not require a free sulfhydryl group for enzymatic activity, showed a *p*H-inhibition curve which was remarkably similar to that of bromelain. These similarities and differences may provide clues to the nature of the active sites on these enzymes, or to the effect of unknown materials in these preparations which mediates the action of diisopropylfluorophosphate on sulfhydryl groups.

That the sulfhydryl group is the site actually being affected is shown by an experiment summarized in Table 1. Blocking the sulfhydryl group with *p*-chloromercuribenzoate before exposing the enzyme to diisopropylfluorophosphate gave complete protection against inhibition. Another sulfhydryl blocking technique, and one which may frequently occur with enzyme preparations, is mild oxidation. This also protected against diisopropylfluorophosphate.

Attempts to reactivate diisopropylfluorophosphate-inhibited bromelain with cysteine, choline, or a combination of the two reagents at either *p*H 5.0 or *p*H 7.0 have been only slightly successful. Cysteine regenerated no more than 10 percent of the original activity.

Our finding that diisopropylfluorophosphate, under the proper conditions, will react with sulfhydryl enzymes now makes this chemical a fairly general protein reagent. It will react directly with the nitrogen of imidazole or the hydroxyl of tyrosine (*4*); it will react directly or indirectly with amino (*5*) and sulfhydryl groups; it will react indirectly with the hydroxyl group of serine (*1, 6*).

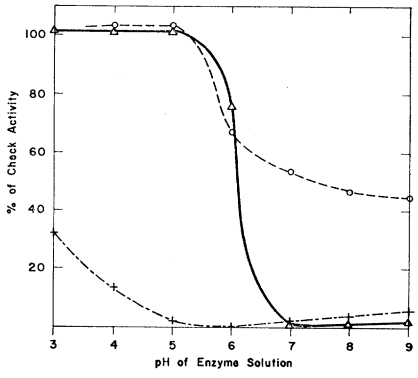


Fig. 1. Effect of *p*H on the inhibition of bromelain (solid line), papain (dashed line with crosses), and Rhozyme P-11 (dashed line with circles) by diisopropylfluorophosphate. The enzymes were incubated for 1 hour at 25°C in $1 \times 10^{-3}M$, $1 \times 10^{-3}M$, and $1 \times 10^{-4}M$ diisopropylfluorophosphate, respectively.

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### References and Notes

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Table 1. Effect of protecting the sulfhydryl group of a purified bromelain sample on inactivation by diisopropylfluorophosphate (DFP).

| Treatment | Milk-clotting unit*/g | Percentage of 4660 |
|---|---|---|
| Enzyme in *p*H 7.0 buffer (no PCMB)† | 4660 | 100 |
| Enzyme in 25 percent isopropylalcohol (IPA) | 4520 | 97 |
| Assayed without cysteine after dialysis | 2620 | 56 |
| Assayed with 0.005*M* cysteine after dialysis | 4500 | 97 |
| Enzyme in 25 percent IPA with $10^{-3}M$ DFP | 706 | 15 |
| Assayed without cysteine after dialysis | 52 | 1 |
| Assayed with 0.005*M* cysteine after dialysis | 450 | 10 |
| Enzyme in *p*H 7.0 buffer with PCMB† | 80 | 2 |
| Enzyme in 25 percent isopropylalcohol | 0 | 0 |
| Assayed without cysteine after dialysis | 552 | 12 |
| Assayed with 0.005*M* cysteine after dialysis | 4900 | 105 |
| Enzyme in 25 percent IPA with $10^{-3}M$ DFP | 0 | 0 |
| Assayed without cysteine after dialysis | 486 | 10 |
| Assayed with 0.005*M* cysteine after dialysis | 4900 | 105 |

* Milk-clotting unit: 1 min to clot 5 ml of a 5-percent skim milk solution adjusted to *p*H 5.3 and incubated at 37.5°C. † *p*-Chloromercuribenzoate.

## Influence of Adrenalectomy and Hypophysectomy on Cerebral Serotonin

*Abstract.* Changes in serous and encephalic serotonin in hypophysectomized or adrenalectomized rats have been observed. Adrenalectomy produces a decrease of serous serotonin and an increase of the serotonin of hemispheres, base, and medulla oblongata; with hypophysectomy, serotonin is also reduced in serum and increased only in the base and medulla oblongata.

It is known that the cerebral serotonin content can be varied by artificial means, either by stimulating the formation of