of waves; cirri are held maximally extended as water, which is often rich in food, sweeps over them. *Lepas,* on the other hand, occurs on freely floating timbers moving *with* the current. The peduncle is highly mobile and swings in every direction; the cirri are more active, though they seldom exhibit the rhythmic beating so characteristic of the balanoids. These motions could be those of foraging, in a situation where the probability of obtaining food by random contact with organisms is greatly reduced (even though at sea certain forms tend to congregate below floating objects).

What is the natural diet of these organisms? Examination of the gut contents from monthly collections of *Mitella* taken over a period of a year showed that copepods, algae, and unidentified particulate matter are nearly always present; cirriped molts, amphipods, cypris larvae, small clams, and hydroids occur frequently; polychaetes and barnacle nauplii are taken occasionally. Batham's list for *Mitella spinosus* is comparable. Polychaetes and plant fragments over one-half the gut length were found coiled inside.

The guts of a number of field specimens of *Lepas* were also inspected, disclosing polychaetes, amphipods, (gammarids, hyperiids, and caprellids), caridean shrimps, gastropods, clams, pycnogonids, algae, and detritus. Some of these items were doubtless captured while the timber bearing the barnacles was washed about in the intertidal zone. The largest crustaceans measured nearly one-half the length of the barnacle gut holding them.

Despite the effective predaceous behavior often exhibited by both *Lepas* and *Mitella,* these forms, as feeders, are opportunistic rather than selective; *Lepas,* attached to timbers stranded on the beach, will fill their guts with sand, while small chunks of granite and calcareous shell have been noted in the gut of *Mitella* (*6*).

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References and Notes

1. C. Darwin, *A Monograph on the Sub-class Cirripedia* (Ray Society, London, 1851).
2. E. J. Batham, *Trans. Proc. Roy. Soc. New Zealand* 74 (1945).
3. F. A. Brown, *Selected Invertebrate Types* (Wiley, New York, 1950).
4. A. Gruvel, *Arch. zool. exptl. et gén.* 1, ser. 3 (1893).
5. C. Nilsson-Cantell, *Zool. Bidr. Uppsala* 7 (1921).
6. We wish to thank Dr. Donald P. Abbott of Hopkins Marine Station for his encouragement and generous advice on this project.

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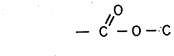
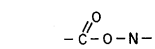
Structural Correlation between Esterase and Protease Activities of Trypsin

Abstract. It is tentatively concluded from ultraviolet and x-ray studies that the two tryptic activities are mediated by overlapping "enzymatic sites." Crucial to this conclusion were studies of the factors which can modify the measured inactivation rates. The data are interpreted in the light of postulated mechanisms of inactivation.

Some enzymes are presumed to have more than one catalytic activity since they act upon substrates that are quite different. Whether these activities are mediated by a single site or a number of separated sites on the enzyme surface is a problem of current biochemical interest and importance. Previous workers concluded that the esterase and protease activities of trypsin (that is, the ability to hydrolyze, respectively, the



structure of esters and the



structure of polypeptides and proteins) must be located at a single site. The evidence is twofold (*1*): (i) Inhibitors of fairly large molecular weight produce equivalent decreases in the two activities, and (ii) trypsin hydrolyzes either type of substrate at a decreased rate in the presence of the other type. However, this evidence does not preclude the existence of closely adjacent or overlapping sites.

The radiation studies reported here were designed to examine this question without use of large-sized inhibitors. Presumably, x-ray inactivation is initiated by radiation-produced radicals of low molecular weight (*2*). Inactivation by ultraviolet irradiation (2537 A) is probably localized within a small region of trypsin, since only one molecule is inactivated per approximately 60 quanta absorbed (each of 4.9 ev). In both studies, the two activities should be inactivated at identical rates if only a single site is involved but probably, although not necessarily, at different rates if adjacent or overlapping sites exist.

The same two activities are inactivated unequally when chymotrypsin is x-irradiated in dilute solution (*3*) or oxidized with sodium periodate (*4*). This has been attributed to concentric sites having different areas or charge configurations (*3*).

While such conclusions are possibly correct, they now appear to require additional justification, since we have found that measured inactivation rates are affected by the treatment afforded irradiated molecules prior to and during assay. Specifically, our experiments are consistent with these postulates: (i) At least three classes of trypsin molecules are present in solution after irradiation—active, damaged, and inactive. (ii) Damaged molecules have an average of one to three more intact H-bonds than do inactive ones and therefore can be converted to the inactive class either by urea ($> 5.5M$) or by thermal treatment prior to the addition of a substrate. (iii) Damaged molecules have normal activity either when only substrate is added or after urea is added if they have been previously exposed to substrate at a *p*H consistent with activity. (iv) This prior exposure to substrate "reactivates" damaged molecules to active ones.

I obtained results similar to those of Aronson, Mee, and Smith (*3*) when 0.4 to 5.0μM solutions of trypsin (twice recrystallized, from Worthington Biochemical Co.) in 0.001M Na_2HPO_4 were irradiated at 0°C with 250 kv (peak) x-rays. Protease activity was determined by hydrolysis of hemoglobin (*5*) (Hb); esterase activity, by hydrolysis of benzoyl-arginine ethyl ester (*6*) (BAEE). The decrease (inactivation) of both activities with increasing dose fitted (± 10 percent) the kinetics of a first-order reaction. The two inactivation rates depend upon the initial trypsin concentration and are quite different. That measured by Hb assay (which employs 5.5M urea) was about 1.5 times that measured by BAEE assay (no urea) when both were extrapolated to infinite solute concentration (*2*). If a one-to-one correspondence between reaction probability and fraction of surface area is assumed, the number of amino acids in the proteolytic site which are reactive to radiation-produced radicals is estimated to be between two and four (*2*).

Only slightly different results were obtained when ultraviolet irradiation instead of x-ray was carried out at 0°C (see plots for H and B, 0° in Fig. 1): the inactivation rates were essentially independent of trypsin concentration between 0.4 and 5.0μM and in a ratio of about 3:1 or 4:1 rather than 1.5:1. The four postulates depend critically upon the following preliminary results with ultraviolet irradiation (see Fig. 1).

1) The rate of inactivation measured by Hb assay appears to be independent of the temperature during irradiation, from 0° to 60°C [see (H, 0–60°)].

2) When samples irradiated at 0° are heated at 60° for 30 minutes (B, 0°:P60°, 30 m) or 100 minutes (B, 0°:P60°, 100 m) immediately after irradiation, and thus prior to assay, the "BAEE-measured rate" approaches the standard "Hb-measured rate."

3) The rate of inactivation measured

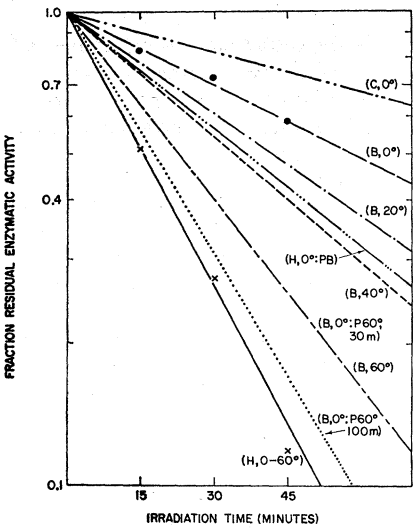


Fig. 1. Effect of temperature, urea, and substrate on the measured level of ultraviolet inactivation of trypsin. The labeling on the curves is explained in the text. Data are shown for only two curves for clarity of presentation; other data showed comparable variances.

by BAEE assay has a ΔH^* of 2500 ± 500 cal/mole from zero (B, 0°) to 60° (B, 60°), approaching the "Hb-measured rate" at 60°C.

4) Without exposure to urea or thermal treatment, an amount of BAEE, which would normally be completely hydrolyzed in 5 minutes, is added in buffer (*p*H 8) 10 to 30 minutes prior to assay; the inactivation rates measured by a subsequent Hb assay are more characteristic of "BAEE-measured rates" than of Hb rates (H, 0°:PB).

The results suggest that radiation causes previously inaccessible H-bonds—necessary for activity—to become available for urea attack or else unstable to the molecular swelling it produces. Thus it appears that the effects of urea or heat, or both, are additive with those of irradiation. This result was predicted previously on the basis of my hypothesis (*7, 8*) that inactivation by physical means proceeds by the sequential rupture of the disulfide and hydrogen bonds making up a "weak-link" structure which is instrumental in "latching" the enzyme together.

The calculated efficiency of 2537-A light in promoting protein inactivation through the rupture of disulfide bonds (*9*) indicates that the data presented here are probably in accord with this hypothesis. Also consistent are the observations of McDonald (*10*) and Monier (*11*) that trypsin molecules having different sensitivities to thermal aging and urea denaturation are produced by x-irradiation of dilute solutions. However, Liener (*12*) recently reported that irreversible trypsin inactivation results from the rupture of only one disulfide bond, rather than my predicted two. This expected correlation between inactivation and increase in —SH titer is now being investigated.

The "reactivation" proposed in the fourth postulate had also been previously anticipated (*8*). However, although my evidence indicates that damaged molecules are reactivated through interaction with BAEE, it is difficult to exclude the possibility that a significant portion of the reversal is produced by the change in *p*H from 4.5 to 8. Probably the reactivation contributions of substrate and *p*H can be best differentiated with a nonproteolytic enzyme, since the possibility of tryptic autodigestion interferes with the interpretation of some of the critical control experiments.

The reversal by BAEE of inactivation which would have been measured by Hb assay indicates that portions of the two activities are probably inactivated by a common mechanism (*13*) (weak link ?). In addition, protease assays employing casein (C, 0°) without urea suggest that the *reactivation capability* differs among substrates. For example, in one experiment the rate of inactivation measured by casein assay (no urea) had a $\Delta H^* = 4750 \pm 500$ cal/mole from 0° to 60°C, as compared with 2500 ± 500 for the BAEE assay.

These and the results cited in paragraphs 2) and 3) above, plus the evidence reviewed in the first paragraph, lead to a tentative conclusion of overlapping sites—where the hydrolytic apparatus would be common but the elements which could form specific attachment would vary with the substrate. Unfortunately, these preliminary results are as yet insufficient for *unequivocal* specification of the architecture of the sites. However, they provide strong evidence concerning some of the steps whereby inactivation proceeds and, therefore, warrant reporting at this time. It is hoped that extension of these studies will permit a specification of the secondary and tertiary structure critical for enzymatic activity as well as define the structural correlation between the two activities.

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References and Notes

1. P. Desnuelle, *Ann. Rev. Biochem.* 23, 55 (1954); G. Schwert, *ibid.* 24, 83 (1955); N. Green and H. Neurath, *The Proteins* (Academic Press, New York, 1954), vol. IIB, pp. 1057–1198.
2. L. Augenstine, *Radiation Research*, in press.
3. D. Aronson, L. Mee, C. Smith, in *Progress in Radiobiology*, J. Mitchell, B. Holmes, C. Smith, Eds. (Oliver and Boyd, Edinburgh, 1955), pp. 61–69.
4. E. Jansen, A. Curl, A. Balls, *J. Biol. Chem.* 189, 671 (1951).
5. M. Anson, *J. Gen. Physiol.* 22, 79 (1938).
6. "Worthington Biochemical Catalog No. 8" (Worthington Biochemical Co., Freehold, N.J., 1957).
7. L. Augenstine, in *Information Theory in Biology*, H. Quastler, Ed. (Univ. of Illinois Press, Urbana, 1953), pp. 119–121; *J. Phys. Chem.* 61, 1385 (1957); in *Information Theory in Biology*, H. Yockey, Ed. (Pergamon, New York, 1956), pp. 287–291.
8. ———, *Information Theory in Biology*, H. Yockey, Ed. (Pergamon, New York, 1956), pp. 103–123.
9. R. Setlow and B. Doyle, *Biochim. et Biophys. Acta* 24, 27 (1957).
10. M. McDonald, *Brit. J. Radiol.* 27, 62 (1954).
11. R. Monier, *Biophys. et Biochim. Acta* 29, 345 (1958).
12. I. Liener, *J. Biol. Chem.* 225, 1061 (1957).
13. Chymotryptic contamination is probably not the explanation of the effects reported here.

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Lack of Abnormal Hemoglobins in Alaskan Eskimos, Indians, and Aleuts

Abstract. In an examination of the blood of 708 Eskimos, 200 Aleuts, and 44 Indians in Alaska for abnormal types of hemoglobin, only normal hemoglobin A was detected. It may be concluded that abnormal hemoglobins in these races are rare if they occur at all.

Although hemoglobins other than normal adult hemoglobin A are found with varying frequency in various racial groups (*1*), these genetic variants of hemoglobin have been found primarily among African or Asian populations. Since the Eskimos, Aleuts, and Indians of Alaska may be of Asian origin, we wished to determine whether any abnormal hemoglobins were characteristic of these Alaskan racial groups. The possibility was also considered that the moderate anemia which is prevalent in Eskimos in western Alaska (*2*) might be related to the presence of an abnormal variant of hemoglobin.

Hemoglobin samples from 593 Eskimos from all parts of Alaska and 25 Indians from central Alaska were tested by paper electrophoresis in Veronal buffer (*p*H 8.6), by alkali denaturation (*3*), and for solubility (*4*). Blood cells from 42 of these persons with moderately low hemoglobins were further examined for osmotic fragility, and the absorption spectra of the hemoglobins were measured. No abnormalities were found in any of the tests.

An additional 334 blood samples were sent to the University of Texas (*5*). Of these, 200 were from Aleuts, 115 were from Eskimos, and 19 were from Indians. The hemoglobins were analyzed by