was directly investigated in disrupted epimastigotes. Figure 2 shows, however, that no catalase activity could be detected in the epimastigote homogenate. The possible existence of catalase inhibitors in the homogenate may be excluded by comparing the activity of beef liver catalase (Sigma Chemical Company; C-100) in homogenate-containing and homogenate-free reaction mixtures, respectively (Figure 2). In contrast to these negative results, peroxidase activity could be demonstrated in the epimastigote homogenate (Figure 2) and also in the particulate fractions obtained therefrom (Table). Peroxidase activity was closely associated with the particulate $480 \times g$ and $680 \times g$ fractions, which included the microbodies (Figure 1B). The non-sedimentable fraction at $105{,}000 \times g$ showed a significant, though lower, peroxidase-specific activity, which might reflect the release of enzyme from microbodies broken during cell fractionation. In the absence of ascorbate, the rate of hydrogen peroxide decomposition by the microbody-containing fractions was negligible (0–3% of the rate in the presence of ascorbate).

Discussion. DAB positivity is standard cytochemical evidence for the identification of catalase-containing microbodies[4–6, 19, 20] but the reaction is also positive with peroxidase[21–23] and cytochrome oxidase[12, 21, 24]. The presence of peroxidase in *T. cruzi* microbodies is in good agreement with a) the preferent distribution of peroxidatic activity in the parasite high density fractions (Table); b) the fact that peroxidases selectively oxidize donors having the enediol structure, such as ascorbate[18] (Figure 2 and Table), and c) the cytochemical demonstration of peroxidase in *T. cruzi* by KALLINICOVA[25]. Participation of cytochrome oxidase in DAB peroxidation may be excluded by the restricted distribution of the electron-opaque material in Figure 1. The apparent absence of catalase in *T. cruzi* microbodies recalls similar negative observations with microbodies from *Trichomonas foetus*[26]. Furthermore, investigation of catalase in other *Trypanosomatidae*[27–29] and *Trichomonas*[30], with methods different from the one employed by us, failed to demonstrate significant amounts of enzyme in these organisms. *T. brucei* has no catalase, but shows peroxidase activity[9].

19 H. D. FAHIMI, J. cell. Biol. *43*, 275 (1969).
20 S. GOLDFISCHER, J. Histochem. *17*, 681 (1969).
21 K. I. HIRAI, J. Histochem. Cytochem. *19*, 434 (1971).
22 M. J. KARNOVSKY, J. Cell. Biol. *27*, 49A (1965).
23 R. C. GRAHAM JR. and M. J. KARNOVSKY, J. Histochem. Cytochem. *14*, 291 (1966).
24 S. GOLDFISCHER, J. cell. Biol. *34*, 398 (1967).
25 V. D. KALLINIKOVA, Acta protozool. *6*, 87 (1968).
26 M. MÜLLER, J. Histochem. Cytochem. *21*, 955 (1973).
27 W. I. STRANGEWAYS, Ann. trop. Med. Parasit. *31*, 405 (1937).
28 S. C. HARVEY, J. biol. Chem. *179*, 435 (1949).
29 J. F. RYLEY, Biochem. J. *59*, 353 (1955).
30 J. F. RYLEY, Biochem. J. *59*, 361 (1955).

Electron-Microscopic Mapping of the Hinge Region of Myosin

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Summary. The trypsin-sensitive sites in the labile hinge region of the myosin molecule are located with heightened accuracy ($\pm$ 2 nm) by electron microscopy as lying at 70, 85, 95, and 103 nm from the C-terminus of the rod section of the molecule.

A restricted region within the rod section of the myosin molecule lying at 70–110 nm from the C-terminus shows special properties including high susceptibility to proteases[3–7] and to thermal denaturation[8]. It has been termed the *hinge* region, and a role in muscle shortening has been proposed[8–10]. The loci must susceptible to tryptic attack have been inferred from the molecular weights of digestion products or determined in the electron microscope[7, 11, 12]. I report here a more accurate mapping of the sites of tryptic attack on myosin that has been aggregated into segment structures or on similar arrays of light meromyosin-C (LMM-C), which is a C-terminal fragment of myosin that is liberated by digestion with BrCN[13], and which contains most of the hinge region. Advantageously, the molecules in the planar arrays are held straight by contact with neighbors, and positions of digested margins can be accurately measured in the electron microscope.

Myosin and LMM-C were prepared[13–15] and aggregates were grown by dialysis-dilution[16] to a final concentration of 0.22 *M* calcium acetate. The resulting myosin aggregates were aligned segments[16], and those with LMM-C were a segment structure, phase F′, with an overlap width of 88 $\pm$ 4 nm and a fringe width of 12 $\pm$ 2 nm (all limits of error are standard deviations). LMM-C was also aggregated by dialysis-dilution from 0.7 *M* to 0.5–0.59 *M* calcium butyrate to give a new segment structure, phase R, with an overlap width of 64 $\pm$ 2 nm and a fringe width of 30 $\pm$ 1 nm.

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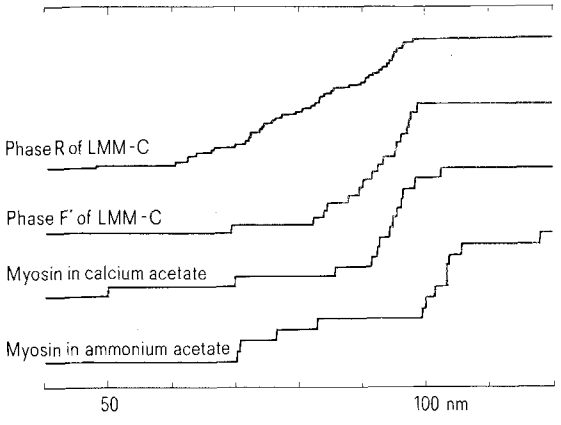
2 I thank Dr. MICHAEL YOUNG for valuable advice and encouragement, Drs. JEROME GROSS and ROMAINE R. BRUNS for use of their electron microscope, and Mrs. MURIEL H. BLANCHARD for excellent technical assistance. This work was supported by the American Heart Association, The John A. Hartford Foundation, Inc., and the National Institutes of Health.

3 E. MIHÁLYI and A. G. SZENT-GYÖRGYI, J. biol. Chem. *201*, 189 (1953).
4 J. GERGELY, M. A. GOUVEA, and D. KARIBIAN, J. biol. Chem. *212*, 165 (1955).
5 W. R. MIDDLEBROOK, Science *130*, 621 (1959).
6 D. R. KOMINZ, E. R. MITCHELL, T. NIHEI, and C. M. KAY, Biochemistry *4*, 2373 (1965).
7 S. LOWEY, H. S. SLAYTER, A. G. WEEDS, and H. BAKER, J. molec. Biol. *42*, 1 (1969).
8 M. BURKE, S. HIMMELFARB, and W. F. HARRINGTON, Biochemistry *12*, 701 (1973).
9 F. PEPE, J. molec. Biol. *27*, 227 (1967).
10 H. HUXLEY, Science *164*, 1356 (1969).
11 E. F. WOODS, S. HIMMELFARB, and W. F. HARRINGTON, J. biol. Chem. *238*, 2374 (1963).
12 D. M. SEGAL, S. HIMMELFARB, and W. F. HARRINGTON, J. biol. Chem. *242*, 1241 (1967).
13 M. YOUNG, M. H. BLANCHARD, and D. BROWN, Proc. natn. Acad. Sci., USA *61*, 1087 (1968).
14 W. W. KIELLEY and L. B. BRADLEY, J. biol. Chem. *218*, 653 (1956).
15 E. G. RICHARDS, C. S. CHUNG, D. B. MENZEL, and H. S. OLCOTT, Biochemistry *6*, 528 (1967).
16 M. V. KING and M. YOUNG, J. molec. Biol. *63*, 539 (1972).

Samples of the aggregates suspended in their dialysis solutions were digested at 20 °C with trypsin in a 1:255 mass ratio to myosin or 1:92.2 to LMM-C. Another sample of myosin segments was transferred to 0.5 *M* ammonium acetate prior to digestion to study the effect of salt composition. Aliquots removed after varying times were quenched with chilled solutions of soybean trypsin inhibitor, and specimens were mounted, negatively stained, and examined in the electron microscope[16].

The Figure summarizes for the different digestions the distances of scission loci from the overlap margins, i.e., the lengths of the residual light-meromyosin fragments.



Cumulative frequency graphs of the distances from the C-termini of the molecules to scission sites in tryptic digestions of segment structures of myosin and LMM-C.

To avoid the artifact-prone grouping of measurements into ranges, the data are plotted as cumulative frequency graphs, rather than as histograms. Each unit step in a broken line corresponds to one datum, and the step heights are inversely proportional to the total number of measurements in each series, so that average slopes reflect the densities of data points.

The digestions of myosin show a single site of predominant attack in each solvent, but the sites differ: 103 ± 2 nm from the C-terminus in ammonium acetate solution, but 95 ± 2 nm in calcium acetate. While very close sites cannot be distinguished, Student's *t*-test suggests that the 95-nm and 103-nm sites differ significantly at better than the 0.1% level. The remaining scission sites for myosin mostly cluster about 70 nm or 85 nm, with one scission at 103 nm in calcium acetate. All of this fits with the observation[7] that brief tryptic digestion of dissolved myosin yields light-meromyosin molecules about 90 nm long, but 70 nm long upon further digestion.

The digestion of phase F′ of LMM-C in calcium acetate largely matches that of myosin in the same solvent, but with less clearly demarcated scission at the 95-nm site, which lies near the N-terminus of LMM-C. Thus the type of solvent is the major factor governing specificity of attack.

In great contrast, the digestion of phase R of LMM-C shows a great loss of specificity of attack. A wide range of sites throughout the hinge region is rapidly cleaved. Thus, calcium butyrate has rendered susceptible many additional sites within the hinge region. This fact, combined with the observation that LMM-C yields ordered, though aberrant aggregates in calcium butyrate solution, suggests that this salt induces an alteration in the LMM-C molecule that is limited to the hinge region.

Anaerobe Aktivierung von Katalase (EC 1.11.1.6) in Gegenwart von Monodehydro-L(+)-ascorbat

Anaerobic Inactivation of Catalase (EC 1.11.1.6) in the Presence of Monodehydro-L(+)-ascorbate

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Summary. Catalase is partially inactivated irreversibly in anaerobic solutions containing together L(+)-ascorbate, monodehydro-L(+)-ascorbate, and didehydro-L(+)-ascorbate. The experiments indicate that monodehydro-L(+)-ascorbate inactivates catalase.

ORR[1–4] hat die lange bekannte unter *aeroben* Bedingungen zur Inaktivierung von Katalase durch L(+)-Ascorbat führende Reaktion auf molekularer Ebene untersucht und zuletzt[2,3] beschrieben, dass unter aerober Inkubation der Katalase mit L(+)-Ascorbat oder L(+)-Ascorbat und Kupferionen (pH 7,0) entstehende Hydroxyl- oder Perhydroxylradikale das Apoprotein der Katalase je nach Bedingungen unterschiedlich verändern. Die Literatur zu diesem Problem ist in den Arbeiten[1–4] ausführlich dargestellt. Bislang ist noch nicht untersucht worden, ob nicht L(+)-Ascorbat, Monodehydro-L(+)-ascorbat oder Didehydro-L(+)-ascorbat, Substanzen, die bei den in der Literatur mitgeteilten Experimenten immer nebeneinander vorliegen, unter *anaeroben* Bedingungen Katalase inaktivieren.

Material und Methoden. Reine kristalline Didehydro-L(+)-ascorbinsäure wurde nach dem Verfahren von STAUDINGER und WEIS[5], Monodehydro-L(+)-ascorbat durch Komproportionierung von L(+)-Ascorbat und Didehydro-L(+)-ascorbat, wie bei VON FOERSTER et al.[6] beschrieben, hergestellt. Extrem reiner Stickstoff der Fa. Messer, Griesheim, wurde mit dem Oxisorbsystem der gleichen Firma nachgereinigt. Wasser wurde deionisiert und zweimal in einer Quarzapparatur destilliert. Die übrigen Chemikalien waren analysenreine Handelsprodukte. Die Vorinkubation der Katalase erfolgte bei 25 °C in einer Stickstoffatmosphäre. Das Schutzgas strömte mit 120 ml · min^{-1} über die in einem Überdruckventil verschlossenen Kolben befindliche Reaktionslösung. Zur Feststellung der katalatischen Aktivität der Katalase bestimmten wir die anfängliche Reaktionsgeschwindigkeit der Zersetzung von Wasserstoffperoxid durch Katalase bei 25 °C. Ausser bei der Untersuchung der pH-Abhängigkeit der Reaktion wurde bei pH 7,0 gearbeitet. Es wurde die Abnahme der Extinktion bei 240 nm Wellenlänge in einem UV-Spektralphotometer SP 800 A mit